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(54) Title: VACCINATION AND METHODS AGAINST DISEASES RESULTING FROM PATHOGENIC RESPONSES BY SPECIFIC T CELL POPULATIONS

(57) Abstract

The present invention provides vaccines and a means of vaccinating a vertebrate so as to prevent or control specific T cell mediated pathologies, including autoimmune diseases and the unregulated replication of T cells. The vaccine is composed of a T cell receptor (TCR) or a fragment thereof corresponding to a TCR present on the surface of T cells mediating the pathology. The vaccine fragment can be a peptide corresponding to sequences of TCRs characteristic of the T cells mediating said pathology. Such a peptide can bind to conventional antigens complexed to MHC antigen presenting cells or to superantigens. Means of determining appropriate amino acid sequences for such vaccines are also provided. The vaccine is administered to the vertebrate in a manner that induces an immune response directed against the TCR of T cells mediating the pathology. This immune response down regulates or deletes the pathogenic T cells, thus ablating the disease pathogenesis. The invention aditionally provides specific β-chain variable regions of T cell receptors, designated Vβ3, Vβ4, Vβ12, Vβ14 and Vβ17, which are associated with the pathogenesis of autoimmune diseases, such as rheumatoid arthritis (RA) and multiple sclerosis (MS). Also provided are means to detect, prevent and treat RA and MS. Methods of administering DNA or RNA encoding the polypeptides useful as vaccines of the present invention into the tissue cells of an individual is also provided.

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VACCINATION AND METHODS AGAINST DISEASES RESULTING FROM PATHOGENIC RESPONSES BY SPECIFIC T CELL POPULATIONS

BACKGROUND OF THE INVENTION

5   This invention relates to the immune system and, more specifically, to methods of modifying pathological immune responses.

           Higher organisms are characterized by an immune system which protects them against invasion by
10 potentially deleterious substances or microorganisms. When a substance, termed an antigen, enters the body, and is recognized as foreign, the immune system mounts both an antibody-mediated response and a cell-mediated response. Cells of the immune system termed B
15 lymphocytes, or B cells, produce antibodies that specifically recognize and bind to the foreign substance. Other lymphocytes termed T lymphocytes, or T cells, both effect and regulate the cell-mediated response resulting eventually in the elimination of the antigen.

20   A variety of T cells are involved in the cell-mediated response. Some induce particular B cell clones to proliferate and produce antibodies specific for the antigen. Others recognize and destroy cells presenting foreign antigens on their surfaces. Certain T cells

regulate the response by either stimulating or
suppressing other cells.

While the normal immune system is closely
regulated, aberrations in immune response are not
5   uncommon. In some instances, the immune system functions
inappropriately and reacts to a component of the host as
if it were, in fact, foreign. Such a response results in
an autoimmune disease, in which the host's immune system
attacks the host's own tissue. T cells, as the primary
10   regulators of the immune system, directly or indirectly
effect such autoimmune pathologies.

Numerous diseases are believed to result from
autoimmune mechanisms. Prominent among these are
rheumatoid arthritis, systemic lupus erythematosus,
15   multiple sclerosis, Type I diabetes, myasthenia gravis
and pemphigus vulgaris. Autoimmune diseases affect
millions of individuals world-wide and the cost of these
diseases, in terms of actual treatment expenditures and
lost productivity, is measured in billions of dollars
20   annually. At present, there are no known effective
treatments for such autoimmune pathologies. Usually,
only the symptoms can be treated, while the disease
continues to progress, often resulting in severe
debilitation or death.

25   In other instances, lymphocytes replicate
inappropriately and without control. Such replication
results in a cancerous condition known as a lymphoma.
Where the unregulated lymphocytes are of the T cell type,
the tumors are termed T cell lymphomas. As with other
30   malignancies, T cell lymphomas are difficult to treat
effectively.

Thus, a long-felt need exists for an effective
means of curing or ameliorating T cell mediated

pathologies. Such a treatment should ideally control the
inappropriate T cell response, rather than merely
reducing the symptoms. The present invention satisfies
this need and provides related advantages as well.

5   Summary of the Invention

              The present invention provides vaccines and a
means of vaccinating a vertebrate so as to prevent or
control specific T cell mediated pathologies. The
vaccine is composed of a substantially pure T cell
10   receptor (TCR) or an immunogenic fragment thereof
corresponding to a TCR present on the surface of T cells
mediating the pathology. The vaccine fragment can be a
peptide corresponding to sequences of TCRs characteristic
of the T cells mediating said pathology.

15   The invention additionally provides specific ß-
chain variable regions and their immunogenic segments,
and in particular three T cell receptors, designated Vß3,
Vß14 and Vß17, which are associated with the pathogenesis
of autoimmune diseases, for example rheumatoid arthritis
20   (RA) and multiple sclerosis (MS). Additional VDJ
junctional (CDR3) regions associated with other
autoimmune diseases are also provided. The present
invention further relates to means for detecting,
preventing and treating RA, MS and other autoimmune
25   diseases.

              The invention further provides methods of
preventing or treating T cell mediated pathologies,
including RA and MS, by gene therapy. In these methods,
pure DNA or RNA encoding for a TCR, an immunogenic
30   fragment thereof or an anti-idiotype antibody having an
internal image of a TCR or an immunogenic fragment is
administered to an individual. Vectors containing the

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DNA or RNA and compositions containing such vectors are
also provided for use in these methods.

Brief Description of the Figures

Figure 1 shows the variable region sequences of
5   Vβ3, Vβ14 and Vβ17. The boxed segments depict the CDR1,
CDR2 and CDR4 hypervariable regions of each Vβ chain.
The sequences between the CDR2 and CDR4 regions represent
an overlap between these two hypervariable regions.

Figure 2(A) shows the location of primers used
10 in polymerase chain reaction amplification of T cell
receptor β-chain genes, and 2(B) shows primer sequences
used in polymerase chain reaction.

Figure 3 shows the location and sequence of
primers used in polymerase chain reaction amplification
15 of HLA-DR B_1 genes. Also shown are HLA-DR allele specific
oligonucleotides.

Detailed Description of the Invention

The invention generally relates to vaccines and
their use for preventing, ameliorating or treating T
20 cell-mediated pathologies, such as autoimmune diseases
and T cell lymphomas. Vaccination provides a specific
and sustained treatment which avoids problems associated
with other potential avenues of therapy.

As used herein, the term "T cell-mediated
25 pathology" refers to any condition in which an
inappropriate T cell response is a component of the
pathology. The term is intended to encompass both T cell
mediated autoimmune diseases and diseases resulting from
unregulated clonal T cell replication. In addition, the
30 term is intended to include both diseases directly

mediated by T cells and those, such as myasthenia gravis,
which are characterized primarily by damage resulting
from antibody binding, and also diseases in which an
inappropriate T cell response contributes to the
5 production of those antibodies.

As used herein, "substantially the amino acid
sequence," or "substantially the sequence" when referring
to an amino acid sequence, means the described sequence
or other sequences having any additions, deletions or
10 substitutions that do not substantially effect the
ability of the sequence to elicit an immune response
against the desired T cell receptor sequence. Such
sequences commonly have many other sequences adjacent to
the described sequence. A portion or segment of the
15 described immunizing sequence can be used so long as it
is sufficiently characteristic of the desired T cell
receptor or fragment thereof to cause an effective immune
response against desired T cell receptors, but not
against undesired T cell receptors. Such variations in
20 the sequence can easily be made, for example by
synthesizing an alternative sequence. The alternate
sequence can then be tested, for example by immunizing a
vertebrate, to determine its effectiveness.

As used herein, the term "fragment" means an
25 immunogenically effective subset of the amino acid
sequence that comprises a TCR. The term is intended to
include such fragments in conjunction with or combined
with additional sequences or moieties, as for example
where the peptide is coupled to other amino acid
30 sequences or to a carrier. The terms "fragment" and
"peptide" can, therefore, be used interchangeably since a
peptide will be the most common fragment of the T cell
receptor. Each fragment of the invention can have an
altered sequence, as described above for the term
35 "substantially the sequence."

Reference herein to a "fragment," "portion" or "segment" of a T cell receptor does not mean that the composition must be derived from intact T cell receptors. Such "fragments," portions" or "segments" can be produced

5 by various means well-known to those skilled in the art, such as, for example, manual or automatic peptide synthesis, various methods of cloning or enzymatic treatment of a whole TCR.

As used herein when referring to the

10 relationship between peptide fragments of the invention and sequences of TCRs, "corresponding to" means that the peptide fragment has an amino acid sequence which is sufficiently homologous to a TCR sequence or fragment thereof to stimulate an effective regulatory response in

15 the individual. The sequence, however, need not be identical to the TCR sequence as shown, for instance, in Examples II and III.

By "immunogenically effective" is meant an amount of the T cell receptor or fragment thereof which

20 is effectively elicits an immune response to prevent or treat a T cell mediated pathology or an unregulated T cell clonal replication in an individual. Such amounts will vary between species and individuals depending on many factors for which one skilled in the art can

25 determine.

As used herein, "binding partner" means a compound which is reactive with a TCR. Generally, this compound will be a Major Histocompatibility Antigen (MHC) but can be any compound capable of directly or indirectly

30 stimulating T cell activation or proliferation when bound to the TCR. Such compounds can also, for example, be a superantigen that binds to a superantigen binding site on the TCR.

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As used herein, "individual" means any vertebrate, including humans, capable of having a T cell mediated pathology or unregulated clonal T cell replication and is used interchangeably with
5　"vertebrate."

As used herein, "ligand" means any molecule that reacts with another molecule to form a complex.

As used herein, "selectively binds" means that a molecule binds to one type of molecule or related group
10　of molecules, but not substantially to other types of molecules. In relation to Vβs, "selective binding" indicates binding to TCRs or fragments thereof containing a specific Vβ without substantial cross-reactivity with other TCRs that lack the specific Vβ.

15　　　The immune system is the primary biological defense of the host (self) against potentially pernicious agents (non-self). These pernicious agents may be pathogens, such as bacteria or viruses, as well as modified self cells, including virus-infected cells,
20　tumor cells or other abnormal cells of the host. Collectively, these targets of the immune system are referred to as antigens. The recognition of antigen by the immune system rapidly mobilizes immune mechanisms to destroy that antigen, thus preserving the sanctity of the
25　host environment.

The principal manifestations of an antigen-specific immune response are humoral immunity (antibody mediated) and cellular immunity (cell mediated). Each of these immunological mechanisms are initiated through the
30　activation of helper (CD4+) T Cells. These CD4+ T cells in turn stimulate B cells, primed for antibody synthesis by antigen binding, to proliferate and secrete antibody. This secreted antibody binds to the antigen and

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facilitates its destruction by other immune mechanisms.
Similarly, CD4+ T cells provide stimulatory signals to
cytotoxic (CD8+) T cells that recognize and destroy
cellular targets (for example, virus infected cells of
5 the host). Thus, the activation of CD4+ T cells is the
proximal event in the stimulation of an immune response.
Therefore, elaboration of the mechanisms underlying
antigen specific activation of CD4+ T cells is crucial in
any attempt to selectively modify immunological function.

10   T cells owe their antigen specificity to the T
cell receptor (TCR) which is expressed on the cell
surface. The TCR is a heterodimeric glycoprotein,
composed of two polypeptide chains, each with a molecular
weight of approximately 45 kD. Two forms of the TCR have
15 been identified. One is composed of an alpha chain and a
beta chain, while the second consists of a gamma chain
and a delta chain. Each of these four TCR polypeptide
chains is encoded by a distinct genetic locus containing
multiple discontinuous gene segments. These include
20 variable (V) region gene segments, joining (J) region
gene segments and constant (C) region gene segments.
Beta and delta chains contain an additional element
termed the diversity (D) gene segment. Since D segments
and elements are found in only some of the TCR genetic
25 loci, and polypeptides, further references herein to D
segments and elements will be in parentheses to indicate
the inclusion of these regions only in the appropriate
TCR chains. Thus, V(D)J refers either to VDJ sequences
of chains which have a D region or refers to VJ sequences
30 of chains lacking D regions.

         With respect to the beta chain of the variable
region referred to as a Vβ, the nomenclature used herein
to identify specific Vβs follows that of Kimura et al.,
<u>Eur. J. Immuno.</u> 17:375-383 (1987), with the exception

that the Vβ14 herein corresponds to Vβ3.3 of Kimura et al.

During lymphocyte maturation, single V, (D) and J gene segments are rearranged to form a functional gene
5   that determines the amino acid sequence of the TCR expressed by that cell. Since the pool of V, (D) and J genes which may be rearranged is multi-membered and since individual members of these pools may be rearranged in virtually any combination, the complete TCR repertoire is
10   highly diverse and capable of specifically recognizing and binding the vast array of binding partners to which an organism may be exposed. However, a particular T cell will have only one TCR molecule and that TCR molecule, to a large degree if not singly, determines the specificity
15   of that T cell for its binding partner.

Animal models have contributed significantly to the understanding of the immunological mechanisms of autoimmune disease. One such animal model, experimental
20   allergic encephalomyelitis (EAE), is an autoimmune disease of the central nervous system that can be induced in mice and rats by immunization with myelin basic protein (MBP). The disease is characterized clinically by paralysis and mild wasting and histologically by a
25   perivascular mononuclear cell infiltration of the central nervous system parenchyma. The disease pathogenesis is mediated by T cells having specificity for MBP. Multiple clones of MBP-specific T cells have been isolated from animals suffering from EAE and have been propagated in
30   continuous culture. After in vitro stimulation with MBP, these T cell clones rapidly induce EAE when adoptively transferred to healthy hosts. Importantly, these EAE-inducing T cells are specific not only for the same antigen (MBP), but usually also for a single epitope on
35   that antigen. These observations indicate that discrete

populations of autoaggressive T cells are responsible for the pathogenesis of EAE.

Analysis of the TCRs of EAE-inducing T cells has revealed restricted heterogeneity in the structure of
5   these disease-associated receptors. In one analysis of 33 MBP-reactive T cells, only two alpha chain V region gene segments and a single alpha chain J region gene segment were found. Similar restriction of beta chain TCR gene usage was also observed in this T cell
10 population. Only two beta chain V region segments and two J region gene segments were found. More importantly, approximately eighty percent of the T cell clones had identical amino acid sequences across the region of beta chain V-D-J joining. These findings confirm the notion
15 of common TCR structure among T cells with similar antigen specificities and indicate that the TCR is an effective target for immunotherapeutic strategies aimed at eliminating the pathogenesis of EAE.

Various attempts have been made to exploit the
20 antigen specificity of autoaggressive T cells in devising treatment strategies for EAE. For example, passive administration of monoclonal antibodies specific for TCRs present on EAE-inducing T cells has been employed. In the mouse model of EAE, infusion of a monoclonal antibody
25 specific for Vβ8, the major beta chain V region gene of by MBP-specific T cells, reduced the susceptibility of mice to subsequent EAE induction as described in Acha-Orbea et al., Cell 54:263-273 (1988) and Urban et al., Cell 54:577-592 (1988). Similar protection has been
30 demonstrated in rat EAE with monoclonal antibody reactive with an unidentified idiotypic determinant of the TCR on MBP specific T cells (Burns et al., J. Exp. Med. 169:27-39 (1989)). While passive antibody therapy appears to have some ameliorative effect on EAE susceptibility, it
35 is fraught with potential problems. The protection

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afforded is transient, thus requiring repeated
administration of the antibody. Multiple infusions of
antibody increase the chances the host will mount an
immune response to the administered antibody,

5   particularly if it is raised in a xenogeneic animal.
Further, an antibody response to a pathogenic T cell
clone represents only one element in the complete immune
response and neglects the potential contributions of
cellular immunity in resolving the autoreactivity.

10   The role of cellular immunity in reducing the
activity of autoaggressive T cells in EAE has been
examined and potential therapies suggested. In a manner
similar to the passive antibody approach, regulatory T
cells have been derived ex vivo and readministered for

15 immunotherapy. For example, a CD8+ T cell line was
recently isolated from convalescing rats in which EAE had
been induced by adoptive transfer of an MBP-specific CD4+
T cell line. Sun et al., Nature, 332:843-845 (1988).
This CD8+ T cell line displayed cytolytic activity in

20 vitro for the CD4+ T cell used to induce disease.
Moreover, adoptive transfer of this CTL line reduced the
susceptibility of recipient rats to subsequent challenge
with MBP. Lider et al., Science, 239:181-183 (1988) also
report the isolation of CD8+ T cells with suppressive

25 activity for EAE-inducing T cells. These CD8+ cells were
isolated from rats vaccinated with attenuated disease-
inducing T cell clones and, though they showed no
cytolytic activity in vitro, they suppressed MBP-driven
proliferation of EAE-inducing T cells. Although these

30 studies indicate that the CD8+ T cells could downregulate
EAE, it is hard to reconcile a major role for these
selected CD8+ CTLs in the long-term resistance of the
recovered rats since Sedgwick, et al., (Eur. J. Immunol.,
18:495-502 (1988)) have clearly shown that depletion of

35 CD8+ cells with monoclonal antibodies does not affect the
disease process or recovery.

In the experiments of Sun et al., and Lider et
al., described above, the administration of _ex vivo_
derived regulatory T cells overcomes the major obstacle
of passive antibody therapy in that it permits a
5   regulatory response _in vivo_ of prolonged duration.
However, it requires _in vitro_ cultivation with attenuated
disease-inducing T cells to develop clones of such
regulatory T cells, a costly and labor intensive process.
Further, in an outbred population such as humans, MHC
10   non-identity among individuals makes this a highly
individualized therapeutic strategy. Regulatory clones
need to be derived for each individual patient and then
re-administered only to that patient to avoid potential
graft versus host reactions.

15   Direct vaccination with attenuated disease-
inducing T cell clones also has been employed as a
therapy for EAE. MBP-specific T cells, capable of
transferring disease, have been attenuated by gamma
irradiation or chemical fixation and used to vaccinate
20   naive rats. In some cases, vaccinated animals exhibited
resistance to subsequent attempts at EAE induction (Lider
et al., _supra_; see Cohen and Weiner, Immunol. Today
9:332-335 (1988) for review). The effectiveness of such
vaccination, however, is inconsistent and the degree of
25   protection is highly variable. T cells contain a
multitude of different antigens which induce an immune
response when the whole T cell is administered as a
vaccine. This phenomenon has been demonstrated by Offner
et al., _J. Neuroimmunol._, 21:13-22 (1989) by showing that
30   immunization with whole T cells increased the delayed
type hypersensitivity (DTH) response, as measured by ear
swelling, to those T cells in an incremental manner as
the number of vaccinations increased. However, positive
DTH responses were found in both protected and non-
35   protected animals. Rats responded similarly to both the
vaccinating encephalitogenic T cells and control T cells.

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Conversely, vaccination with PPD-specific T cells from a
PPD-specific T cell line induced DTH to the vaccinating
cells as well as to an encephalitogenic clone even though
no protection was observed. The similar response of

5 vaccinated rats to both disease-inducing and control
cells, as quantified by delayed-type hypersensitivity (a
measure of cell-mediated immunity), indicates that
numerous antigens on these T cells are inducing immune
responses. Thus, vaccination with attenuated disease-

10 inducing T cells suffers from a lack of specificity for
the protective antigen on the surface of that T cell, as
well as, variable induction of immunity to that antigen.
As a candidate for the treatment of human diseases,
vaccination with attenuated T cells is plagued by the

15 same labor intensiveness and need for individualized
therapies as noted above for infusion of CD8+ cells.

An alternative mechanism for T cell activation
has been suggested in which endogenous and exogenous
superantigens have been shown to mediate T-cell

20 stimulation as described, for example, in White et al,
Cell 56:27-35 (1989) and Janeway, Cell 63:659-661 (1990).

As used herein, "superantigens" means antigens
or fragments thereof that bind preferentially to T cells
at specific sites on the β chain of a TCR and stimulate T

25 cells at very high frequency rate. Such superantigens
can be endogenous or exogenous. "Frequency" refers to
the proportion of T cells responding to antigens and
ranges from about 1/5 to 1/100 in response to
superantigens. Thus, superantigens are distinguishable

30 from conventional antigens, which have a much lower T
cell response frequency rate ranging from about $1/10^4$ to
$1/10^6$. Superantigens activate T cells by binding to
specific Vβs. The superantigen binding sites of various
TCRs have been distinguished from the conventional

35 hypervariable regions (CDRs) of TCRs. These CDRs

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represent the regions of TCRs thought to be responsible for binding conventional antigens that are complexed to MHC.

5   The present invention provides an effective method of immunotherapy for T cell mediated pathologies, including autoimmune diseases, which avoids many of the problems associated with previously suggested methods of treatment. By vaccinating, rather than passively administering heterologous antibodies, the host's own

10 immune system is mobilized to suppress the autoaggressive T cells. Thus, the suppression is persistent and may involve any or all immunological mechanisms in effecting that suppression. This multi-faceted response is more effective than the uni-dimensional suppression achieved

15 by passive administration of monoclonal antibodies or _ex vivo_-derived regulatory T cell clones which requires a highly individualized therapeutic approach because of MHC non-identity among humans in order to avoid graft versus host reactions. The methods of the present invention are

20 also more effective than vaccination with attenuated disease-inducing T cells that lack specificity for the protective antigen on the surface of a particular T cell as well as the variable induction of immunity to that antigen. In addition, vaccination with attenuated T

25 cells is plagued by the same labor intensiveness and need for individualized therapies as noted above for _ex vivo_ derived regulatory T cell clones.

     As they relate to autoimmune disease, the vaccine peptides of the present invention comprise TCRs

30 or immunogenic fragments thereof from specific T cells that mediate autoimmune diseases. The vaccines can be whole TCRs substantially purified from T cell clones, individual T cell receptor chains (for example, alpha, beta, etc.) or portions of such chains, either alone or

35 in combination. The vaccine can be homogenous, for

example, a single peptide, or can be composed of more than one type of peptide, each of which corresponds to a different portion of the TCR. Further, these peptides can be from different TCRs that contribute to the T cell

5   mediated pathology. These vaccine peptides can be of variable length so long as they can elicit a regulatory response. Preferably, such peptides are between about 5 - 100 amino acids in length, and more preferably between about 6 - 25 amino acids in length.

10   In a specific embodiment, the immunizing peptide can have the amino acid sequence of a ß-chain VDJ region when the subject has MS or RA. Any immunogenic portion of these peptides can be effective, particularly a portion having substantially the sequence SGDQGGNE (SEQ

15   ID No. 1) or CAIGSNTE (SEQ ID No. 2) of Vß4 and Vß12, respectively, for MS or substantially the sequence ASSLGGAVSYN (SEQ ID No. 3), ASSLGGEETQYF (SEQ ID No. 4), ASSLGGFETQYF (SEQ ID No. 5) or ASSLGGTEAFF (SEQ ID No. 6) or RA. Thus, amino acid substitutions can be made which

20   do not destroy the immunogenicity of the peptide. Additionally, this peptide can be linked to a carrier to further increase its immunogenicity. Alternatively, whole T cell receptors or TCR fragments that include these sequences can be used to vaccinate directly.

25   In a further specific embodiment, T cell receptors, whole T cells or fragments of TCRs that contain Vß17, Vß14 or Vß3 can be used to immunize an individual having a T cell mediated pathology to treat or prevent the disease. In a specific embodiment,

30   rheumatoid arthritis can be so treated. The immune response generated in the individual can neutralize or kill T cells having Vß17, Vß14 or Vß3 and, thus, prevent or treat the deleterious effects of such Vß-bearing T cells. Moreover, to the extent that Vß17, Vß14 or Vß3 is

35   common to T cell receptors on pathogenic T cells

mediating other autoimmune diseases or autoimmune diseases in general, such vaccines can also be effective in ameliorating such other autoimmune diseases.

5   As used herein, "Vβ17" refers to a specific human β-chain variable region of three T cell receptors. Vβ17 has the following amino acid sequence: MSNQVLCCVVLC FLGANTVDGGITQSPKYLFRKEGQNVTLSCEQNLNHDAMYWYRQDPGQGLRLIYYSQ IVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASS (SEQ ID No. 7).

10   "Vβ14" refers to a specific human β-chain variable regions of another TCR. Vβ14 has the following amino acid sequence: MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITV TGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSRKEK RNFPLILESPSPNQTSLYFCASS (SEQ ID No. 8).

15   "Vβ3" refers to a family of specific human β-chain variable region. Two members of the Vβ3 family have been identified as Vβ3.1 and Vβ3.2. Vβ3.1 has the following amino acid sequence: MGIRLLCRVAFCFLAVGLVDVKV TQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYFSYDVKMKEKGDIP
20   EGYSVSREKKERFSLILESASTNQTSMYLCASS (SEQ ID No. 9). Vβ3.2 has the following amino acid sequence: MGIRLLCRVAFC FLAVGLVDVKVTQSSRYLVKRTGEKVFLECVDMDHENMFWYQRQDPGLGLRLIYF

The hypervariable or junctional regions are useful for the vaccines of the present invention.
25 Hypervariable regions useful in the present invention include CDR1, CDR2, CDR3 and CDR4. The amino acid sequences of the CDR1, CDR2 and CDR4 hypervariable regions for Vβ3, Vβ14 and Vβ17 are shown in Figure 1.

The CDR3, also known as the V(D)J region, is useful as a vaccine of the present invention since T cell immunity elicited by peptides corresponding to this region is expected to be highly specific for a particular
5   antigen. Due to the recombination of the V, D and J region genes prior to maturation, the amino acid sequence across these regions is virtually unique to each T cell and its clones.

However, as a germ-line element, the CDR2
10   region is also useful in human diseases such as MS and in particular RA.   In RA studies, the results indicate a limited number of Vβs among the activated T cells infiltrating the synovial target tissue with only a few incidences of sequence homology in the βVDJ region.
15   Thus, peptides corresponding to the CDR2 region are viable alternatives for use as vaccines of the present invention. For example, the CDR2 region of Vβ3, DPGLGLRLIYFSYDVKMKEKG (SEQ ID No.75), of Vβ14, DPGLGLRQIYYSMNVEVTDKG (SEQ ID No.76), or of Vβ17,
20   DPGQGLRLIYYSQIVNKFQKG (SEQ ID No.77), can be used.

Modifications in these sequences that do not affect the ability of the receptor or an immunogenic fragment thereof to act as an immunogen to stimulate the desired immune response are contemplated and are included
25   in the definition of TCR fragment. The variable region can be joined with any D and J segment of the TCR. Further, immunogenically representative fragments of Vβ3, Vβ14 and Vβ17 are also included in the definition of "Vβ3," "Vβ14" and "Vβ17," respectively.

30   By "substantially pure," it is meant that the TCR is substantially free of other biochemical moieties with which it is normally associated in nature. Such substantially pure TCRs or fragments thereof, for instance, can be synthesized, produced recombinantly by

means known to those skilled in the art. In addition, whole TCRs can be enzymatically treated to produce such fragments.

In another embodiment, vaccine peptides can
5   correspond to the Vß regions that contain sequences of high homology which are conserved among pathogenic TCRs. These regions of conserved homology include the conventional CDRs, such as CDR1 and CDR2, which are common to T cells bearing the same Vß, and also the
10   superantigen binding site, which can be common to pathogenic TCRs bearing different Vßs. The superantigen binding site is also known to be in or around the CDR4 hypervariable region.

The vaccines of the present invention comprise
15   peptides of varying lengths corresponding to the TCR or immunogenic fragments thereof. The vaccine peptides can correspond to regions of the TCR which distinguish that TCR from other nonpathogenic TCRs. Such specific regions can, for example, be located within the various region(s)
20   of the respective TCR polypeptide chains, for example, a short sequence spanning the V(D)J junction, thus restricting the immune response solely to those T cells bearing this single determinant.

The vaccines are administered to a host
25   exhibiting or at risk of exhibiting an autoimmune response. Definite clinical diagnosis of a particular autoimmune disease warrants the administration of the relevant disease-specific TCR vaccines. Prophylactic applications are warranted in diseases where the
30   autoimmune mechanisms precede the onset of overt clinical disease (for example, Type I Diabetes). Thus, individuals with familial history of disease and predicted to be at risk by reliable prognostic indicators

could be treated prophylactically to interdict autoimmune, mechanisms prior to their onset.

TCR peptides can be administered in many possible formulations, including pharmaceutically
5   acceptable mediums. In the case of a short peptide, the peptide can be conjugated to a carrier, such as KLH, in order to increase its immunogenicity. The vaccine can include or be administered in conjunction with an adjuvant, of which several are known to those skilled in
10 the art. After initial immunization with the vaccine, further boosters can be provided. The vaccines are administered by conventional methods, in dosages which are sufficient to elicit an immunological response. Such dosages can be easily determined by those skilled in the
15 art.

Appropriate peptides to be used for immunization can be determined as follows. Disease-inducing T cell clones reactive with the target antigens are isolated from affected individuals. Such T cells are
20 obtained preferably from the site of active autoaggressive activity such as a lesion in the case of pemphigus vulgaris, the central nervous system (CNS) in the case of multiple sclerosis or the synovial fluid or tissue in the case of rheumatoid arthritis.
25 Alternatively, such T cells can be obtained from blood of affected individuals. The TCR genes from these autoaggressive T cells are then sequenced. Polypeptides corresponding to TCRs or portions thereof that are selectively represented among disease inducing T cells
30 (relative to non-pathogenic T cells) can then be selected as vaccines and made and used as described above. An alternative method for isolating pathogenic T cells is provided by Albertini in PCT Publication No. WO88/10314, published on December 29, 1988.

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Alternatively, the vaccines can comprise anti-idiotypic antibodies which are internal images of the peptides described above. Methods of making, selecting and administering such anti-idiotype vaccines are well

5   known in the art. See, for example, Eichmann, et al., CRC Critical Reviews in Immunology 7:193-227 (1987), which is incorporated herein by reference.

In a further aspect of the present invention, methods of preventing the proliferation of T cells

10 associated with a T cell mediated pathology are also contemplated. Such methods include determining a T cell receptor binding partner according to the above methods and administering an effective amount of such binding partner in an appropriate form to prevent the

15 proliferation of the T cells. The methods can be used, for example, to build a tolerance to self antigens as in the case of an autoimmune disease.

The present invention also relates to other methods of preventing or treating a T cell pathology by

20 inhibiting the binding of a T cell receptor to its TCR binding partner in order to prevent the proliferation of T cells associated with the T cell pathology. Ligands that are reactive with the T cell receptor or its binding partner at binding sites that inhibit the T cell receptor

25 attachment to the binding partner can be used. Such ligands can be, for example, antibodies having specificity for the T cell receptor or its binding partner.

The invention also provides a method of

30 preventing or treating a T cell mediated pathology in an individual comprising cytotoxically or cytostatically treating a Vß-containing T-cells, particularly Vß3, Vß14 and Vß17, in the individual. The Vß-containing T cells are treated with a cytotoxic or cytostatic agent that

selectively binds to the Vβ region of a T cell receptor
that mediates a pathology, such as RA or MS for example.
The agent can be an antibody attached to a radioactive or
chemotherapeutic moiety. Such attachment and effective
5   agents are well known in the art. See, for example,
Harlow, E. and Lane, Antibodies, A Laboratory Manual,
Cold Spring Harbor Laboratory (1988), which is
incorporated herein by reference.

Rheumatoid Arthritis

10   Rheumatoid arthritis (RA) is a T cell mediated
autoimmune disease. The invention describes clonal
infiltrates of activated Vβ3, Vβ14 and Vβ17 T cells in
the synovium of rheumatoid arthritis patients. The
presence of these T cells in the diseased tissue of most
15   of patients examined, their clonality, and the cytotoxic
activity of one such T cell for synovial adherent cells,
demonstrate a central role for T cells bearing these Vβs
in the pathogenesis of RA.

         Activated T cell populations in the synovial
20   tissue of RA patients have been examined by analyzing T
cell receptor (TCR) mRNAs isolated from IL-2 receptor
positive (IL-2R+) synovial T cells. As described in
Example X(C), TCR mRNAs were amplified using a polymerase
chain reaction (PCR) protocol designed to amplify human
25   TCR β-chain genes containing virtually any desired Vβ
gene element. In this analysis, clonal Vβ17
rearrangements were found to be enriched in the IL2-R+
population, indicating that Vβ17 T cells are likely
involved in the pathogenesis of RA. A CD4+, Vβ17 bearing
30   T cell clone has been isolated from one of the synovial
tissue specimens and its in vitro cytotoxicity for
synovial adherent cells supports the direct involvement
of Vβ17 T cells in RA.

22

Additional studies were conducted to ensure that the prevalence of Vβ17 T cells in the initial studies did not result from an amplification bias for the Vβ consensus primer, and to examine the involvement of

5 other TCR Vβ gene families in RA. As described in Example XI, RNAs from activated (IL2-R+) synovial T cells were analyzed by PCR-amplification with a panel of Vβ-specific PCR primers. In this analysis, Vβ17 transcripts were found in four of the five patients tested,

10 confirming the association of Vβ17 with RA and validating the utility of the Vβ consensus primer. In addition, Vβ14 was found in four of the five RA patient samples and Vβ3 and Vβ9 were detectable in three of five patients.

The sequences of these various Vβ polypeptides

15 were examined for homology to Vβ17. The results are reported in Table 1.

TABLE 1

Relative Homologies of TCR β Chain Polypeptides With Human Vβ17

hVβ	100%	(94aa)
mVβ6	69.1%	(94aa)
hVβ3	58.5%	(94aa)
hVβ12.1	53.2%	(94aa)
hVβ14	52.1%	(94aa)
mVβ7	51.7%	(89aa)
hVβ9	33.0%	(94aa)

hVβ = human Vβ
mVβ = mouse Vβ

30 As shown in Table 1, mouse Vβ6 is most closely homologous, followed by hVβ3, hVβ12.1, hVβ14, and mVβ7. Three of the human Vβs, Vβ3, Vβ14 and Vβ17, were detected in the synovium of RA patients. Vβ12.1 was negligible in the synovium despite considerable overall homology with

Vβ17. In contrast, Vβ9 was found in three of five synovial samples, yet is only weakly homologous to Vβ17.

A surprising discovery is the greater homology found among all of the Vβs detected in the synovia of RA
5 patients, except Vβ9, in a contiguous stretch of 15 amino acids located carboxy to the CDR2 region. The 15 amino acid sequences of these Vβs as well as other human and mouse Vβs are shown in Table 2. Within the β-chain, this region of conservation corresponds positionally to that
10 previously shown to contain superantigen binding sites.

TABLE 2

Proposed Superantigen Binding Site in RA Associated Vβ Genes

		%H[1]	Seq. ID. No.
15 hVβ17	+- EGYSVS<u>RE</u>KKESFPL		11
hVβ3	+- EGYSVS<u>RE</u>KKERFSL	86.7	12
20 hVβ14	+- EGYKVS<u>RKE</u>KRNFPL	66.7	13
hVβ12.1	DGYSVSRSKTEDFLL	66.7	14
hVβ9	NRFSPKSPDKAHLNL	<30	15
25 mVβ6	+- EGYDAS<u>RE</u>KKSSFSL	73.3	16
mVβ7	+- KGYRVSRKK<u>RE</u>HFSL	64.3	17
30 mVβ8.2a	DGYKASRPSQENFSL		18
mVβ8.2c	+- ....<u>KE</u>...		19
hVβ13.2	DGYNVSRLKKQNFLLGLE		20

35 [1]%H = % homology compared with Vβ17

The exogenous superantigen, SEC$_2$, stimulates
human Vβ13.2 T cells as a result of binding to a site on
Vβ13.2 as described in Choi et al., _Nature_ 346:471-473
(1990), which is incorporated herein by reference. The
5   sequence of this binding site is shown in Table 2 as the
last 11 amino acids for Vβ13.2.

A binding site for Mls-1a, an endogenous
superantigen, has also been mapped to this region as
described in Pullen et al., _Cell_ 61:1365-1374 (1990),
10 which is incorporated herein by reference.
Identification of this region as the Mls binding site
involved the study of Vβ8.2a, the Vβ8.2 isoform common to
laboratory mice, and Vβ8.2c, a β-chain found in wild
mice. These β-chain polypeptides are distinguished
15 functionally by their differential reactivities with Mls-
1a and structurally by a difference of five amino acids.
Of particular importance are the residues at position 70
and 71. The responder β-chain, Vβ8.2c, has lysine and
glutamic acid, respectively, at these positions.
20 Specific mutagenesis of the non-responder gene to encode
a lysine-glutamic acid pair at positions 70 and 71
rendered that non-responder β-chain Mls-1a-reactive.
This confirms the region as one of superantigen binding.
Thus, both these exogenous and endogenous superantigens
25 can bind in the vicinity of the 15 amino acid sequence
homology identified in Table 2. In addition, the lysine-
glutamic acid pair charge motif is implicated in Mls-1a
reactivity. Involvement of this charge motif in Mls-1a
reactivity is confirmed by its presence in mouse Vβ6 and
30 Vβ7, two other Mls-1a reactive murine β-chains. The
Vβ8.2c, Vβ6 and Vβ7 charge motif of a lysine or arginine
followed by a glutamic acid residue is underlined in
Table 2. Thus, the superantigen binding site for Mls-1a
is characterized by this charge motif contained within
35 the region of local homology.

25

The present invention is directed to the unexpected discovery that human Vβ3, Vβ14 and Vβ17 have a region that corresponds to the Mls-1a binding site. These three human Vβs display a significant degree of

5   overall homology within the entire 94 amino acid sequence and of local homology within the 15 amino acid sequence with mVβ6 and mVβ7. Each of these Vβs possess a lysine or arginine/glutamic acid pair, which are underlined in Table 2 and represent what is meant by the term "charge

10 motif." Vβ12.1, while displaying a high degree of overall and local homology with Vβ3, Vβ14 and Vβ17, lacks the charge motif, perhaps accounting for its presence in the synovium of only one of five RA patients. Vβ9 shows no overall or local homology to Vβs 3, 14 or 17 and lacks

15 the charge motif.

The presence of Vβ3, Vβ14 and Vβ17-bearing T cells has been demonstrated among the activated synovial T cells in RA. These three β-chain polypeptides, in contrast to other known Vβs, possess overall and local

20 sequence homology and an apparent superantigen binding charge motif. These results indicate that Vβ-specific T cell activation by superantigen plays a role in RA.

Vβ3, Vβ14 and Vβ17 are the only known human Vβ chains to possess this apparent superantigen binding site

25 characterized by this local sequence homology and the identified charge motif. However, it is possible that other Vβs may become known that contain such binding sites. Thus, a substantially pure Vβ3, Vβ14 or Vβ17 sequence containing the charge motif can be used as an

30 immunogen in the vaccines of the present invention. For example, the sequences or fragments thereof shown in Table 2 for Vβ3, Vβ14 and Vβ17 can be used. Vaccines containing any combination of these three Vβ sequences, including all three sequences, can be used effectively to

35 ameliorate T cell associated diseases.

26

In addition, other common V(D)J sequences of the ß-chain observed in RA patients are listed in Table 3. The results taken from two different RA studies show sequence homologies in the ßVDJs from four different
5   clones, which indicates the usefulness of peptides corresponding to the CDR3 region as appropriate vaccine candidates.

TABLE 3

ß-Chain VDJ Sequences Found in Common in RA Patients

10

Patient	Vß	VDJ Sequence	Jß	Seq. ID No.
1012	Vß14	A S S L G G A V S - Y N	Jß2.1	3
1013	Vß3	A S S L G G E E T Q Y F	Jß2.5	4
C	Vß3	A S S L G G F E T Q Y F	Jß2.5	5
15 A	Vß3	A S S L G G T E A - F F	Jß1.1	6

As noted, the invention provides the discovery that specific variable regions of the ß-chains of three TCRs, designated Vß3, Vß14, and Vß17, are closely associated with T cell mediated pathologies, especially
20 rheumatoid arthritis in human subjects. This discovery allows for the detection, prevention and treatment of rheumatoid arthritis using the methodology set out in this invention. Similar therapeutic approaches set out above for EAE can be applied to rheumatoid arthritis by
25 those skilled in the art.

Specifically, the invention provides a method of diagnosing or predicting susceptibility to T cell mediated pathologies in an individual comprising detecting T cells having the ß-chain variable region from
30 Vß3, Vß14 or Vß17 in a sample from the individual, the presence of abnormal levels of such Vß-containing T cells

indicating the pathology or susceptibility to the pathology. The Vβ-containing T cells can be qualitatively or quantitatively compared to that of normal individuals. Such diagnosis can be performed, for

5 example, by detecting a portion of the Vβs that does not occur on non-rheumatoid arthritis associated β-chain variable region T-cell receptors. The Vβs of the present invention can be detected, for example, by contacting the Vβs with a detectable ligand capable of specifically

10 binding to the individual Vβs. Many such detectable ligands are known in the art, e.g. an enzyme linked antibody. Alternatively, nucleotide probes, complementary to the individual Vβ of interest, encoding nucleic acid sequences can be utilized to detect such Vβ-

15 containing T cells, as taught, for instance, in Examples X and XI.

The invention also provides a method of preventing or treating a T cell mediated pathology comprising preventing the attachment of a Vβ3-, Vβ14- or

20 Vβ17-containing T-cell receptor to its binding partner. In one embodiment, attachment is prevented by binding a ligand to Vβ3, Vβ14 or Vβ17. In an alternative embodiment, attachment is prevented by binding a ligand to the Vβ3, Vβ14 or Vβ17 binding partner. Attachment can

25 be prevented by known methods, e.g. binding an antibody to the individual Vβs or to its binding partner in order to physically block attachment.

Multiple Sclerosis

T cells causative of multiple sclerosis (MS)
30 have not previously been identified, though MBP-reactive T cells have been proposed to play a role due to the clinical and histologic similarities between MS and EAE. In rat and mouse models of EAE, MBP-reactive, encephalitogenic T cells show striking conservation of β-

chain V(D)J amino acid sequence, despite known
differences in MHC restriction and MBP-peptide antigen
specificity. One embodiment of the invention is premised
on the observation that a human myelin basic protein

5 (MBP)-reactive T cell line, derived from an MS patient,
has a TCR ß-chain with a V(D)J amino acid sequence of Vß4
homologous with that of ß-chains from MBP-reactive T
cells mediating pathogenesis in experimental allergic
encephalomyelitis (EAE), an animal model of MS, as shown

10 in Table 4. This finding demonstrates the involvement of
MBP-reactive T cells in the pathogenesis of MS and
demonstrates that TCR peptides similar to those described
herein for the prevention of EAE can be appropriate in
treating MS. As shown in Table 4, a VDJ sequence of

15 Vß12, CAIGSNTE (SEQ ID No. 2) and one of Vß4, SGDQGGNE
(SEQ ID No. 1), have been observed in MS patients.

TABLE 4

ß-Chain VDJ Sequences in MS Patients
Homologous to Rat VDJ Sequences

Vß	VDJ Sequence	SEQ ID Nos.
RAT Vß8.2	S S D S S N T E	21
RAT Vß8.2	S S D S G N T E	22
HUMAN Vß4	S G D Q G G N E	1
HUMAN Vß12	C A I G S N T E	2

25   The activated cells of one MS patient from the
CSF were analyzed and found to be predominantly Vß14 and
Vß3. In both cases, there was a predominate clone of
Vß14 and a predominate clone of Vß3. These findings
indicate that the results from the RA studies relating to

30 the same Vßs can be extended to other autoimmune
pathologies, including MS.

In this regard, the invention is directed to
the discovery that ß-chain VDJ fragments homologous to
VDJ sequences found in rodent EAE, such as SGDQGGNE (SEQ
ID No.1) and CAIGSNTE (SEQ ID No. 2), are closely

5   associated with multiple sclerosis in human subjects.
This discovery allows for the detection, prevention and
treatment of multiple sclerosis using the methodology set
out in this invention. Similar therapeutic approaches
set out herein for EAE can be applied to multiple

10   sclerosis by those skilled in the art.

Specifically, the invention provides a method
of diagnosing or predicting susceptibility to multiple
sclerosis in an individual comprising detecting T cells
having various Vßs, such as Vß4 or Vß12, and particularly

15   having substantially the sequence SGDQGGNE (SEQ ID No.1)
or CAIGSNTE (SEQ ID No.2), in a sample from the
individual, the presence of the sequence indicating
multiple sclerosis or susceptibility to multiple
sclerosis. The sequences can be detected, for example,

20   by contacting T cells or TCRs with a detectable ligand.
Many such ligands are known in the art, for example, an
enzyme linked or otherwise labeled antibody specific for
the sequence. Alternatively, nucleotide probes
complementary to the nucleic acid encoding the sequence

25   can be utilized as taught, for instance, in Example IX.

The invention also provides a method of
preventing or treating multiple sclerosis comprising
preventing the attachment of a T-cell receptor containing
various Vßs, including Vß4, Vß12 or fragments thereof,

30   such as those having substantially the SGDQGGNE (SEQ ID
No.1) or CAIGSNTE (SEQ ID No.2) sequence to its binding
partner. In one embodiment, attachment is prevented by
binding a ligand to the sequence. In an alternative
embodiment, attachment is prevented by binding a ligand

35   to the binding partner. Attachment can be prevented by

known methods, such as binding an antibody to these Vβs, and in particular to the SGDQGGNE (SEQ ID No.1) or CAIGSNTE (SEQ ID No.2) sequences, to physically block attachment.

5   The invention also provides a method of preventing or treating multiple sclerosis in an individual comprising cytotoxically or cytostatically treating T cells containing various Vβs, including Vβ4, Vβ12 and fragments thereof, particularly those having
10 substantially the SGDQGGNE (SEQ ID No.1) or CAIGSNTE (SEQ ID No.2) sequence in the individual. In one embodiment, T-cells are treated with a cytotoxic or cytostatic agent which selectively binds to these Vβs or their immunogenic fragments. The agent can be, for example, an antibody
15 attached to a radioactive or chemotherapeutic moiety.

T Cell Pathologies of Malignant Etiology

To illustrate the utility of TCR vaccination, autoimmune disease has been discussed. However, T cell lymphoma is another T cell pathology
20 which would be amenable to this type of treatment. Application of this technology in the treatment of T lymphoma would be conducted in virtually identical fashion. In one respect, however, this technology is more readily applied to T cell proliferative disease
25 since the isolation of the pathogenic T cells is more easily accomplished. Once the clones are isolated, the technology is applied in the manner described herein. Specifically, the TCR genes of the T lymphomas are sequenced, appropriate regions of those TCRs are
30 identified and used as vaccines. The vaccines can comprise single or multiple peptides, and can be administered in pharmaceutically acceptable formulations, with or without adjuvants, by conventional means.

31

Gene Therapy

The present invention further relates to an
alternative method of treating or preventing a T cell
mediated pathology by gene therapy. In this method, a
5   nucleic acid encoding for a TCR or an immunogenic
fragment thereof is first inserted into an appropriate
delivery system, for example a plasmid. The nucleic acid
can be DNA or RNA encoding for TCRs, immunogenic
fragments thereof or anti-idiotype antibodies that can be
10 used as vaccines in the present invention. Such DNA or
RNA can be isolated by standard methods known in the art.
The isolated nucleic acid can then be inserted into a
suitable vector by known methods. Such methods are
described, for example, in Maniatis et al., Molecular
15 Cloning: A Laboratory Manual (Cold Spring Harbor
Laboratory 1982), which is incorporated herein by
reference.

The vector is subsequently administered
directly into a tissue of an individual. Preferably, the
20 DNA or RNA-containing vector is injected into the
skeletal muscle of the individual. For example, a 1.5 cm
incision can be made to expose the quadricep muscles of
the subject. A 0.1 ml solution containing from 10-100 μg
of a DNA or RNA plasmid and 5-20% sucrose is injected
25 over 1 minute into the exposed quadricep muscles about
0.2 cm deep. The skin is thereafter closed. The amount
of DNA or RNA plasmid can range from 10 to 100 μl of
hypotonic, isotonic or hypertonic sucrose solutions or
sucrose solutions containing 2 mM $CaCl_3$. The plasmid
30 containing solutions can also be administered over a
longer period of time, for example, 20 minutes, by
infusion. The _in vivo_ expression of the desired gene can
be tested by determining an increased production of the
encoded polypeptide by the subject according to methods

known in the art or as described, for example, in Wolff
et al., Science 247:1465-1468 (1990).

5   It is believed that the treated cells will
respond to the direct injection of DNA or RNA by
expressing the encoded polypeptide for at least about 60
days. Thus, the desired TCR, immunogenic fragment or
anti-idiotype antibody can be effectively expressed by
the cells of the individual as an alternative to
vaccinating with such polypeptides.

10   The present invention also relates to vectors
useful in the gene therapy methods and can be prepared by
methods known in the art. Compositions containing such
vectors and a pharmaceutically acceptable medium are also
provided. The pharmaceutically acceptable medium should
15 not contain elements that would degrade the desired
nucleic acids.

       The following examples are intended to
illustrate but not limit the invention.

EXAMPLE I
RAT MODEL OF EAE

20

       Female Lewis rats, (Charles River Laboratories,
Raleigh-Durham, NC) were immunized in each hind foot pad
with 50 μg of guinea pig myelin basic protein emulsified
in complete Freund's adjuvant. The first signs of
25 disease were typically observed 9-11 days post-
immunization. Disease severity is scored on a three
point scale as follows: 1=limp tail; 2=hind leg
weakness; 3=hind leg paralysis. Following a disease
course of approximately four to six days, most rats
30 spontaneously recovered and were refractory to subsequent
EAE induction.

33

EXAMPLE II

SELECTION AND PREPARATION OF VACCINES

Vaccinations were conducted with a T cell
receptor peptide whose sequence was deduced from the DNA
5   sequence of a T cell receptor beta gene predominating
among EAE-inducing T cells of B10.PL mice. The DNA
sequence was that reported by Urban, et al., supra, which
is incorporated herein by reference. A nine amino acid
peptide, having the sequence of the VDJ junction of the
10 TCR beta chain of the mouse, was synthesized by methods
known to those skilled in the art. The sequence of this
peptide is: SGDAGGGYE (SEQ ID No.23). (Amino acids are
represented by the conventional single letter codes.)
The equivalent sequence in the rat has been reported to
15 be: SSD-SSNTE (SEQ ID No. 24) (Burns et al., J. Exp.
Med. 169:27-39 (1989)). The peptide was desalted by
Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, NJ)
column chromatography in 0.1 M acetic acid and the
solvent was subsequently removed by two cycles of
20 lyophilization. A portion of the peptide was conjugated
to keyhole limpet hemocyanin (KLH) with glutaraldehyde at
a ratio of 7.5 mgs of peptide per mg of KLH. The
resulting conjugate was dialyzed against phosphate
buffered saline (PBS).

25   ## EXAMPLE III

VACCINATION AGAINST EAE

Vaccines used in these studies consisted of
free VDJ peptide and also of VDJ peptide conjugated to
KLH. These were dissolved in PBS and were emulsified
30 with equal volumes of either (1) incomplete Freund's
adjuvant (IFA) or (2) complete Freund's adjuvant (CFA)
made by suspending 10 mg/ml heat killed desiccated
Mycobacterium tuberculosis H37ra (Difco Laboratories,
Detroit, MI) in IFA. Emulsions were administered to 8-12

34

week old female Lewis rats in a final volume of 100
microliters per animal (50 μl in each of the hind
footpads). 5 μg of unconjugated VDJ peptide were
administered per rat. KLH-VDJ conjugate was administered
at a dose equivalent to 10 μg of KLH per rat. Twenty-
nine days later each rat was challenged with 50 μg of
guinea pig myelin basic protein in complete Freund's
adjuvant in the front footpads. Animals were monitored
daily beginning at day 9 for clinical signs of EAE and
were scored as described above. The results are
presented in Table 5. As can be seen, not only was there
a reduced incidence of the disease in the vaccinated
individuals, but in those which did contract the disease,
the severity of the disease was reduced and/or the onset
was delayed. The extent of protection varied with the
vaccine formulation, those including CFA as the adjuvant
demonstrating the greatest degree of protection.

TABLE 5

Animal No.	Vaccination (Adjuvant)	Days After Challenge								
		10	11	12	13	14	15	16	17	18
1	VDJ (IFA)	-	-	2	3	3	3	-	-	-
2	"	-	-	1	3	3	3	2	-	-
3	"	-	-	-	3	3	3	2	-	-
4	VDJ (CFA)	-	-	-	-	1	1	1	-	-
5	"	-	-	-	-	-	-	-	-	-
6	"	-	-	-	1	3	3	3	2	-
7	KLH-VDJ (CFA)	-	-	-	1	3	2	-	-	-
8	"	-	-	-	-	1	1	1	1	-
9	"	-	-	-	-	-	-	-	-	-
10	KLH-VDJ (IFA)	-	1	3	3	2	2	1	-	-
11	"	-	-	3	3	3	3	3	2	-
12	"	-	-	1	3	3	3	3	-	-
13	NONE	1	3	3	3	3	1	-	-	-
14	"	-	1	3	3	3	1	-	-	-
15	"	1	3	3	3	1	-	-	-	-

Scoring: – no signs
        1) limp tail
        2) hind leg weakness
        3) hind leg paralysis

35

EXAMPLE IV

Vaccination against EAE with Lewis Rat VDJ peptides

The VDJ peptide used in the previous examples
was synthesized according to the sequence of TCR β chain
5   molecules found on EAE-inducing T cells in B10.PL mice.
In addition, peptides were synthesized and tested which
correspond to sequences found on encephalitogenic T cells
in Lewis rats. These VDJ sequences are homologous with
that of B10.PL mice, but not identical. The rat peptides
10 were synthesized according to the DNA sequences reported
by Burns, et al. and Chluba, et al., Eur. J. Immunol.
19:279-284 (1989). The sequences of these peptides
designated IR1, 2, 3 and 9b are shown below, aligned with
the B10.PL mouse sequence used in Examples I through III
15 (VDJ):

		SEQ ID No.
VDJ	S G D A G G Y E	23
IR1	C A S S D - S S N T E V F F G K	25
IR2	C A S S D - S G N T E V F F G K	26
IR3	C A S S D - S G N - V L Y F G E G S R	27
IR9b	A S S D - S S N T E	28

The preparation, administration and evaluation
of these vaccines were conducted as described in Examples
I through III with the following exceptions: 50 μg of
25 the individual VDJ peptides were incorporated into
vaccine formulations containing CFA; neither vaccinations
in IFA nor vaccinations with peptides conjugated to KLH
were conducted. Control animals were untreated prior to
MBP challenge as in Example III or were vaccinated with
30 emulsions of PBS and CFA to assess the protective effect
of adjuvant alone. The results are shown in Table 6
below.

TABLE 6

	Animal No.	Vaccination (Adjuvant)	Days After Challenge								
			10	11	12	13	14	15	16	17	18
5	1	None	-	1	2	3	3	2	-	-	-
	2	"	1	3	3	3	2	-	-	-	-
	3	"	-	2	3	3	3	1	-	-	-
	4	PBS-CFA	1	2	3	3	3	-	-	-	-
	5	"	1	2	3	3	3	-	-	-	-
10	6	"	-	2	3	3	3	-	-	-	-
	7	IR1 (50 µg)	-	-	-	2	1	-	-	-	-
	8	"	-	-	-	-	1	3	-	-	-
	9	"	-	-	-	1	1	1	1	-	-
	10	IR2 (50µg)	-	-	1	3	3	3	-	-	-
15	11	"	-	-	-	-	2	2	3	3	-
	12	"	-	-	-	-	1	-	-	-	-
	13	IR3 (50µg)	1	3	3	3	2	-	-	-	-
	14	"	-	-	2	3	3	-	-	-	-
	15	"	-	-	-	-	-	-	-	-	-
20	16	IR9b (50 µg)	-	-	-	-	-	-	-	-	-
	17	"	-	-	-	-	-	-	-	-	-
	18	"	-	-	-	-	-	-	-	-	-
	19	"	-	-	-	-	-	-	-	-	-

25 Scoring: -   no signs
                1)   limp tail
                2)   hind leg weakness
                3)   hind leg paralysis

As shown in Table 6, disease in unvaccinated
30 control animals was observed as early as day 10. Disease
was characterized by severe paralysis and wasting,
persisted for 4 to 6 days and spontaneously remitted.
PBS-CFA vaccinated rats displayed disease courses
virtually indistinguishable from those of unvaccinated
35 controls. In contrast, delays in onset were observed in
some of the IR1, 2 or 3 vaccinated animals and others
showed both delayed onset as well as decreased severity
and/or duration of disease. Overall, however,
vaccinations with the rat VDJ peptides (IR1-3) were
40 slightly less effective than those with the mouse VDJ
peptide (Example III). Vaccination with IR9b, however,
afforded complete protection in all four animals in which
it was tested. Importantly, no histologic lesions
characteristic of disease were found in any of the four

animals vaccinated with IR9b indicating that sub-clinical
signs of disease were also abrogated.

EXAMPLE V
Vaccination with V region specific peptides

5   A peptide specific for the Vβ8 gene family was
tested as a vaccine against EAE. Vβ8 is the most common
β chain gene family used by encephalitogenic T cells in
both rats and mice. A peptide was synthesized based on a
unique DNA sequence found in the Vβ8 gene, and which is
10   not found among other rat Vβ genes whose sequences were
reported by Morris, et al., Immunogenetics 27:174-179
(1988). The sequence of this Vβ8 peptide, designated
IR7, is:

        IR7   DMGHGLRLIHYSYDVNSTEK   (SEQ ID No.29)

15   The efficacy of this Vβ8 peptide was tested in
the Lewis rat model of EAE (Example I) as described in
Examples II and III. 50 µg of peptide were tested in
CFA. Vaccinations in IFA or with peptide-KLH conjugates
were not conducted. The results of these studies are
20   shown in Table 7.

TABLE 7

Animal No.	Vaccination (Adjuvant)	Days After Challenge								
		10	11	12	13	14	15	16	17	18
1	IR7 (50 µg)	–	–	1	2	3	3	3	–	–
2	"	–	–	–	–	1	1	–	–	–
3	"	–	–	–	–	–	–	–	–	–

Scoring: –   no signs
          1)   limp tail
          2)   hind leg weakness
          3)   hind leg paralysis

EXAMPLE VI

Comparison of Vβ8.2 Peptide Lengths

The results of vaccinations conducted with the
rat Vβ8 peptide are similar to those observed with the
5 mouse and rat IR1, 2 and 3 peptides. Delayed onset as
well as decreased severity and duration of disease was
observed in one animal. One animal was completely
protected.

It has been found that a corresponding 21 amino
10 acid sequence of Vβ8.2 (residues 39-59) provided less
protection than IR7 as shown in Table 8. The 21 amino
acid sequence of Vβ8.2 is DMGHGLRLIHYSYDVNSTEKG (SEQ ID
No.30).

39

TABLE 8

Comparison of Efficacy of Two β-Chain CDR2 Peptides
in Protecting from EAE in the Lewis Rats (50)

Vaccination	#With Disease #Tested	Mean Max Severity	Mean Onset	Mean Duration	#With Histology #Tested	Mean histology Score	Mean S.L. (14 days)
None	10/10	2.8	10.5	8.4	10/10	3.7	---
Control Peptide/CFA	9/10	2.8	11.5	8.1	9/9	2.6	1.0
Vβ8.2$_{20}$ CFA	6/10	1.3	11.2	3.8	8/10	1.0	16.4
PBS/CFA	9/10	2.5	12.1	6.0	9/10	2.6	---
Vβ8.2$_{21}$ CFA	6/6	3.0	12.0	6.7	6/6	3.3	6.3

40

        Each peptide was used at 100 μg doses and
dissolved in saline prior to being emulsified in an equal
volume of complete Freund's adjuvant (CFA). Animals were
challenged after 42 days with 50 μg of guinea pig myelin
5   basic protein in CFA. The CFA contained 10 mg/ml of
mycobacteria tuberculosis. Injections and evaluation of
clinical signs and histology were performed as previously
described. Other animals (five per group) were immunized
with peptides in the same way and their splenocytes were
10 removed after 14 days to test for lymphocyte
proliferation as described in Olee et al., J.
Neuroimmunol. 21:235-240 (1989). The sequences of the 20
amino acid peptide and the 21 amino acid peptide are
designated in Table 8 as $V\beta 8.2_{20}$ and $V\beta 8.2_{21}$ respectively.

15   EXAMPLE VII

            Vaccination with J region peptides

        A peptide was synthesized which corresponds to
the J α gene segment, TA39, found among both rat and
mouse encephalitogenic T cell receptors. The sequence of
20 this peptide, designated IR5, is:

            IR5   RFGAGTRLTVK   (SEQ ID No.31)

        The efficacy of the JαTA39 peptide was tested
in the Lewis rat model of EAE (Example I) as described in
Examples II and III. 50 μg of peptide were tested in
25 CFA. Vaccinations in IFA or with peptide-KLH conjugates
were not conducted. The results of these studies are
shown in Table 9.

TABLE 9

Animal No.	Vaccination (Adjuvant)	Days After Challenge										
		10	11	12	13	14	15	16	17	18	19	20
1	IR5 (50 µg)	-	-	-	-	-	2	1	1	1	1	-
2	"	-	-	-	-	-	-	-	-	-	-	-
3	"	-	-	-	-	-	-	-	-	-	-	-

Scoring: -   no signs
          1)   limp tail
          2)   hind leg weakness
          3)   hind leg paralysis

        The results of vaccinations conducted with the
rat J α TA39 peptide are more effective than those
observed with the mouse VDJ peptide or the Vβ8 peptide.
Two of three animals were totally protected and, in the
third, disease onset was markedly delayed. Severity was
also reduced in this animal though disease persisted for
a normal course of 5 days. Importantly, the two animals
which were completely protected showed no histologic
evidence of T cell infiltration of the CNS. This result
indicates that vaccinating with the J_αTA39 very
efficiently induces a regulatory response directed at
encephalitogenic T cells. Even sub-clinical signs of
disease were abrogated.

EXAMPLE VIII

Vaccination with mixtures of TCR peptides

        Vaccinations were conducted with a mixture of
TCR peptides. This mixture contained 50 µg of each of
the peptides IR1, 2, 3 and 5 (the three rat VDJ peptides
and the rat JαTA39 peptide).

        The efficacy of this peptide mixture was tested
in the Lewis rat model (Example I) as described in

Examples II and III. Peptides were tested in CFA.
Vaccinations in IFA or with peptide-KLH conjugates were
not conducted. The results of these studies are shown in
Table 10.

TABLE 10

Animal No.	Vaccination (Adjuvant)	Days After Challenge								
		10	11	12	13	14	15	16	17	18
4	IR1, 2, 3, 5	-	-	-	-	-	-	-	-	-
5	(50 μg each)	-	-	-	-	-	-	-	-	-
6	"	-	-	-	-	-	-	-	-	-

Scoring: -   no signs
            1)   limp tail
            2)   hind leg weakness
            3)   hind leg paralysis

The results of vaccinations conducted with the
rat JαTA39 and three VDJ peptides were as effective as
those described for IR9b in Table 6. All three animals
were totally protected. In addition to the absence of
any clinical signs of EAE, two of these three animals
were completely free of histological evidence of T cell
infiltration into the CNS while the third showed only two
small foci of lymphocytic infiltration at the base of the
spinal cord.

EXAMPLE IX
Multiple Sclerosis Vaccine

A. Human MBP-reactive T cells

MBP-reactive T cell lines were established from
peripheral blood mononuclear cells (PBMC) of nine chronic
progressive MS patients and two healthy controls. Cells
were maintained in culture by regular stimulation with
purified human MBP and irradiated-autologous PBMC for

43

three days followed by four days in IL-2 containing medium.

B.   PCR Amplification of TCR β-chain genes from MBP-reactive T cell lines

5   T cells were harvested from log phase cultures and RNA was prepared, amplified with the Vβ16mer primer and nested Cβ primers for 55 cycles as described in Example X.

C.   TCR β-chain sequences of human MBP-reactive T cells

10   Vβ16mer amplified TCR β-chain genes from human MBP-reactive T cell lines were sequenced using the Cβseq primer. Amplification products were gel purified, base denatured and sequenced from the Cβseq primer. Readable DNA sequence was obtained from 5 of these lines,
15   indicating that predominant T cell clones had been selected by long term in vitro passage. One of these sequences, from the MS-Re cell line (Table 11), possessed a β-chain VDJ amino acid sequence that shared five of the first six and six of nine total residues with the β-chain
20   VDJ amino acid sequence conserved among MBP reactive, encephalitogenic T cells in the B10.PL mouse model of EAE. This sequence was not present among the predominant TCR rearrangements found in the remaining four human MBP reactive T cell lines.

25   To determine if similar sequences were present in the β-chain repertoire of the MBP-reactive T cell lines from other MS patients, PCR amplification was conducted with a degenerate (n=1024) 21-nucleotide primer (VβRe) corresponding to seven amino acids of this
30   sequence. RNAs were reversed transcribed and amplified in 20 cycle stage I reactions with the Vβ16mer and Cβext primers. One μl aliquots of these stage I reactions were

reamplified for 35 cycles with the VβRe and Cβ int

primers. One μl aliquots of these reactions were

analyzed by Southern blot hybridization with a ^{32}P-labeled

human Cβ probe. This analysis revealed the 300 bp

5 amplified product in the Re cell line and in one of the

other MS patient lines, but not in MBP-reactive T cells

from control subjects or in non-MBP reactive human T cell

lines and clones. The presence of this sequence in two

of the nine MS patient lines tested is compelling. Since

10 this sequence is known to be conserved among

encephalitogenic T cells in EAE, its detection among MBP-

reactive T cells from MS patients demonstrates a role for

T cells bearing this determinant in the pathogenesis of

MS.

15   Immunogenic peptides having the sequence

SGDQGGNE (SEQ ID No.1) can be synthesized as shown in

Example II and used to immunize human subjects by methods

demonstrated in Example III. Such immunizations can

result in an effective immune response.

TABLE 11

20

			Vβ	Dβ		Jβ	SEQ ID No.
A)	Sample						
25			Vβ4.2			Jβ2.1	
	MS-Re	ctctgc	agcggagaccagggcggc		aatgagcagttcttc	32
			S G D Q G G -		N E Q F F	33
	B10.PL			S G D A G G G Y E		
23							

30 B)

```
              A              A    A              34
              C      C     A  C    C    C   A     35
      5' G G  G A  C A  G G  G G  A A  G A  3'
              G      T     G  G    G    T   G     36
              T              T    T              37
```

35

EXAMPLE X
Detection of Clonal Infiltrates of
Activated Vß17 T Cells in the Synovium of
Rheumatoid Arthritis Patients

5 **A. T cell preparations from synovial tissue**

Synovial tissue specimens were obtained from
radiographically proven rheumatoid arthritis patients
undergoing joint replacement therapy. Activated T cells
were selected using magnetic beads and antibodies
10 reactive with the human IL2-R (αIL2-R) as follows.
Synovial tissue was digested for 4 hrs at 37°C in RPMI +
10% Fetal Bovine Serum (FBS) containing 4 mg/ml
collagenase (Worthington Biochemical, Freehold, NJ) and
0.15 mg/ml DNAse (Sigma, St. Louis, MO.). Digests were
15 passed through an 80-mesh screen and single cells were
collected by Ficoll density gradient centrifugation.
Cells at the interface were washed and were incubated at
10^6/ml for 30 min at 0°C with 5 μg/ml control mouse IgG
(Coulter Immunology, Hialeah, FL) in PBS containing 2%
20 FBS (PBS-FBS). Cells were washed three times and
incubated for 30 min at 0°C with magnetic beads conjugated
to goat anti-mouse IgG (Advanced Magnetics, Cambridge,
MA). Beads were magnetically separated and washed three
times with PBS-FBS. This preselection with mouse IgG
25 (mIgG) and magnetic beads was used to control for non-
specific adsorption of T cells. The cells remaining in
the initial suspension were further incubated 30 minutes
at 0°C with 5 μg/ml monoclonal mouse IgG reactive with the
human T cell IL2-R (Coulter Immunology, Hialeah, FL).
30 Cells were washed and selected with magnetic beads as
above. Beads from the IgG preadsorption and the IL2-R
antibody selection were immediately resuspended in
acidified-guanidinium-phenol-chloroform and RNA prepared
as described in Chonezynski and Sacchi, Anal. Biochem.

46

162:156 (1987), which is incorporated herein by
reference. Since RNAS were prepared without in vitro
culture of the cells and the accompanying bias that may
be induced, they are expected to accurately reflect T
5 cell distributions in synovial tissue at the time of
surgical removal. Only half of the mIgG and αIL2-R beads
from patient 1012 were immediately processed for RNA.
The remainder were cultured for 5 days in RPMI 1640, 5%
FBS, 20% HL-1 (Ventrex Laboratories Inc., Portland, ME),
10 25mM HEPES, glutamine, antibiotics and 20% LAK
supernatant (Allegretta et al., Science, 247:718 (1990)),
which is incorporated by reference herein, as a source of
IL-2. RNA was extracted from cultures of the αIL2-R
beads (1012IL2.d5), but not from the 1012mIgG sample as
15 no viable cells were present at the end of the 5 day
culture.

A T cell clone was derived from the Ficoll
pellet of patient 1008. The cells in the pellet were
cultured at 2×10^6/ml in media without IL-2 for two
20 weeks. Non-adherent cells from this culture were cloned
by limiting dilution onto autologous synovial cell
monolayers. A CD4+ T cell clone 1008.8 was obtained and
adapted to culture by regular stimulation with autologous
synovial monolayers for 3 days in media without IL-2
25 followed by a 4 day culture in medium with LAK
supernatant.

B. Lysis of Synovial Adherent Cells by 1008.8

Lysis of synovial adherent cells by 1008.8 was
demonstrated as follows. Synovial cell monolayers were
30 labeled as described in Stedman and Campbell, J. Immunol.
Meth. 119:291 (1989), which is incorporated herein by
reference, with ^{35}S for use as targets in CTL assays.
Cells were typsinized, washed and plated at 2000 cells
per well of a 96-well round bottom microtiter plate.

1008.8 cells, cultured for 3 days prior to the assay with synovial adherent cells and medium containing LAK supernatant, were added to the targets at the indicated effector:target ratios. Cultures were incubated

5   overnight at 37°C, centrifuged at 300xg for 2 minutes and radioactivity in 50 μl of the supernatant quantified. Per cent specific lysis was calculated relative to detergent-lysed targets by standard formulas. This clone is cytotoxic for synovial adherent cell targets in CTL

10   assays (Table 12).

TABLE 12

Effector:Target Ratio	% Specific Lysis
5:1	7
10:1	16
25:1	32

C. PCR Amplification of TCR β-chain genes

TCR β-chain genes were amplified with several combinations of the primers shown in Figure 2. The vβ16mer primer is a degenerate Vβ primer (n=256) which is

20   predicted to bind 85% of human TCR β-chain genes at all 16 residues and 95% at 15 residues. This primer has been used to amplify TCR β-chains from more than 25 different human T cell clones, lines or primary tissue preparations. A spectrum of Vβ genes has been sequenced

25   from these amplified DNAs, arguing against a significant bias of the primer for certain Vβ families. Thus, PCR amplification with the Vβ16mer primer facilitates analysis of T cell populations for which a priori knowledge of Vβ gene usage is unavailable.

30

T cell receptor β-chain genes were amplified in two-stage amplification reactions with nested pairs of

the primers shown in Figure 2. The primer sequences used
in the polymerase chain reactions are listed in Table 13.

TABLE 13

5	Vβcons	5' T TC TGGTA G AC CAAA CA 3'	(SEQ ID NO. 47) (SEQ ID NO. 48) (SEQ ID NO. 49)
	Vβ17	5' TCACAGATAGTAAATGACTTTCAG 3'	(SEQ ID NO. 50)
	Vβ8	5' TCTCCACTCTGAAGATCC 3'	(SEQ ID NO. 51)
	Vβ12	5' GATTTCCTCCTCACTCTG 3'	(SEQ ID NO. 52)
10	5'Cβ	5' CAAGCTGTTCCCACCCGA 3'	(SEQ ID NO. 53)
	Cβext	5' CCAGAAGGTGGCCGAGAC 3'	(SEQ ID NO. 54)
	Cβint	5' GCGGCTGCTCAGGCAGTA 3'	(SEQ ID NO. 55)
	Cβseq	5' CGACCTCGGGTGGGAACA 3'	(SEQ ID NO. 56)

The middle line of the Vβcons primer reads: T TT TCGT.

RNAs were reverse transcribed for 1 hour at $42°C$
15 with 40pmol of the Cβext primer in a 12 μl reaction using
conditions described by Hart et al., The Lancet, p. 596
(1988), included by reference herein. Reactions were
diluted with a master mix containing 40 pmols of the
Vβ16mer primer, nucleotides and reaction buffer as above
20 but without $MgCl_2$ to give a final Mg^{+2} concentration of
3.6 mM. Samples were denatured for 15 minutes at $95°C$, 1
unit of heat stable recombinant DNA polymerase (Cetus
Corporation, Emeryville, CA, Ampli-taq™) was added and 20
cycles of PCR conducted. Each cycle consisted of a 1 min
25 denaturation at $95°C$, a two minute annealing step and a
two minute extension at $72°C$. The first two cycles were
annealed at $37°C$ and $45°C$, respectively, and the remainder
at $50°C$. One microliter aliquots of these stage I
reactions were added to 100 μl stage II amplification
30 reactions (Cetus, Gene-Amp Kit™) containing 100 pmols of
the Cβint primer and 100 pmols of the Vβ8, Vβ17 or 5'Cβ
primers or 700 pmols of the Vβ16mer primer. Stage II

amplifications were conducted as above with a 50°C
annealing temperature and without the 37°C and 45°C
ramping.

RNA samples from 1012IL2.d5 and 1008.8 cultures
5   were amplified with the Vβ16mer and CβExt primers in
stage I reactions and with the Vβ16mer and the CβInt
primer in 35 cycle stage II reactions. Reaction
products, purified from low melting agarose gel slices
with Gene Clean glass beads (Bio 1D1, San Diego, CA),
10 were base denatured and sequenced from the CβSeq primer
with T7 polymerase (Sequenase, United States Biochem,
Cleveland, OH). A predominate Vβ sequence, corresponding
to a single Vβ17 rearrangement (Table 14), was clearly
readable in the 1012IL2.d5 sample. Other, less frequent
15 rearrangements were detected as faint, uninterpretable
background bands in the sequencing gels. Culture of
these 1012.IL2 beads in IL2-containing medium without
added accessory cells or antigen is not expected to
induce de novo activation of T cells. Thus, the
20 predominance of a single Vβ17 rearrangement in this
sample reflects in vivo clonal expansion of Vβ17+ T cells
in this patient. DNA sequence determination of TCR β-
chain DNA amplified from the cytotoxic T cell clone,
1008.8, also revealed a Vβ17 rearrangement (Table 14).
25 The presence of Vβ17 rearrangements in these two
different types of synovial T cell samples, derived from
two separate RA patients, implicates Vβ17 bearing T cells
in the pathogenesis of RA.

50

TABLE 14

Sample	Vβ	Dβ	Jβ	SEQ ID NO.
1012 day 5	Y L C A S tatctctgtgccagt Vβ17	K N P T V S aaaaatcccacggtctcc	Y G Y T F tatggctacaccttc Jβ1.2	38 39
1008.8	Y L C A S tatctctgtgccagt Vβ17	D N E S gacaacgagagt	F F G Q G ttctttggacaaggc Jβ1.1	40 41
1014 IL-2	Y L C A S tatctctgtgccagt Vβ17	V R D R R gtgagggacaggaga	N Y G Y T aactatggctacacc Jβ1.2	42 43
1015 IL-2	Y L C A S S tatctctgtgccagtagt Vβ17	S I D S agtatagactcc	S Y E Q Y tcctacgagcagtac Jβ2.7	44 45

To determine whether or not Vβ17 rearrangements were present in the other magnetic bead RNA preparations, TCR β-chain genes were amplified with a Vβ17-specific primer in the second stage amplification after an initial amplification with the Vβ16mer. Vβ17 TCR DNA could be amplified from magnetic bead samples derived from the 4 patients examined. Ethidium bromide staining of electrophoresed reaction products revealed greater Vβ17 amplification in some of the IL-2R+ samples than in the corresponding controls. Accordingly, the relative amounts of Vβ17 TCRs in each control and IL-2R+ sample were quantified by slot blot hybridization analysis as follows.

RNAs from magnetic bead preps were amplified in the first stage with the Vβ16mer and Cβext primers and

then reamplified for twenty cycles with the CBint primer
and each of the VB17, BB8 and 5'CB primers.
Amplification reactions were serially diluted in 20X SSC,
denatured by boiling and chilled in an ice slurry.

5 Samples were loaded onto nitrocellulose membranes,
hybridized to a human TCR B-chain constant region probe
and washed with 0.1X SSC, 0.1% SDS at 56°C. Bound
radioactivity was quantified by liquid scintillation
spectroscopy and endpoint dilutions were those samples

10 with fewer than 200 cpm bound. The amounts of product
produced by forty total cycles with each of the
respective primer combinations falls in the linear
portion of a product versus cycle number quantification
curve.

15   Amplifications with the 5'CB and CBint primer
pair were used to estimate the total B-chain amplified
from each sample, providing benchmarks for normalizing
the results of VB17 and VB8 quantification in the
respective IL-2R+ and control sample pairs (Table 15).

20 The quantity of VB17 DNA amplified was increased in the
IL-2R+ samples, relative to the control samples, in 3 of
the 4 patients. The magnitude of the increase ranged
from 5-fold in patient 1015 to 40-fold in patient 1014
(Table 15). This enrichment was not a product of the

25 isolation procedure, since the quantity of VB8 DNA
amplified was increased in the IL-2R+ fraction only in
patient 1015.

TABLE 15

Sample	Endpoint Dilution CB	VB17	VB8	VB17/CB	VB17 IL-2R/mIgG	VB8/CB	VB8 IL-2/mIgG
1	3,125	3,125	625	1	25	0.2	1
2	3,125	125	625	0.04		0.2	
3	15,625	25	625	0.001	0.12	0.04	0.04
4	3,125	25	3,125	0.008		1	
5	15,625	625	125	0.04	40	0.008	0.04
6	3,125	5	625	0.001		0.2	
7	15,625	625	15,625	0.04	5	1	25*
8	78,125	625	3,125	0.008		0.04	

Sample 1 = 1012 IL-2R+, Sample 2 = 1012 mIgG,
Sample 3 = 1013 IL-2R+, Sample 4 = 1013 mIgG,
Sample 5 = 1014 IL-2R+, Sample 6 = 1014 mIgG,
Sample 7 = 1015 IL-2R+, Sample 8 = 1015 mIgG.

*In a follow-up study in which VB8 was sequenced, it was found that the enrichment of this VB was an artifact and that the value is ≤ 1.

VB17 rearrangements from the IL-2R+ RNAs of the three patients showing enrichment were amplified with the VB17 and CBint primer pair and the reaction products sequenced with the CBseq primer. As was shown for sample 1012 IL-2.d5, 1014 and 1015 contained single sequences (Table 14), indicative of clonal expansion of VB17 T cells in vivo. In contrast, direct sequencing of the rearrangements amplified with the VB8 specific primer was not possible due to significant heterogeneity in the B-chain product.

D. HLA-DR Analysis in Rheumatoid Arthritis Patients

HLA-DR analysis in rheumatoid arthritis patients was performed as follows. DNA from each patient was prepared by boiling 10^5 synovial cells in 200 μl dH$_2$O.

5   Ten μl were amplified for 35 cycles in a 100 μl reaction (Cetus, Gene Amp Kit™) containing 100 pmols of each of the DRß PCR primers shown in Table 16 as DRß1 and DRß3. One-tenth μl of this reaction was reamplified in 10 μls containing only the DRß2 primer and 17 pmol of α32P-dCTP

10 as the sole source of dCTP for 10 cycles. Reactions were spiked with 200 μM dCTP and chased for 2 cycles.   The resulting negative strand probes were hybridized to slot blots containing 10 pmol of the HLA-DR allele specific oligos (positive strands) using conditions previously

15 described by Amar et al., J. Immunol. 138:1947 (1987), which is incorporated herein by reference. The slots were washed twice for 20 minutes with tetramethylammoniumchloride (Wood et al., Proc. Natl. Acad. Sci. USA 82:1585 (1985)) which is incorporated

20 herein by reference) at 65-68°C and exposed to X-ray film.

Each of the patients in this study possessed at least one allele of the HLA-DR genes, DR4w4, DR1, DR4w14 or DR4w15, that are known to predispose for RA (Table

25 16). Also shown in Table 16 are HLA-DR allele specific oligonucleotides.

TABLE 16

57

DRβ1   5' G A G T   C T G G A A C A G C 3'   58
          A   59
          C

(DRβ1 above: A/C alternative above the first A)

60

DRβ2   5' G T A G T T G   T T C T G C A 3'   61
             A   62
             G

(DRβ2 above: A/G alternative on the T position)

HLA-DR ALLELE-SPECIFIC OLIGONUCLEOTIDES

DRβ1 Genes

											SEQ ID NO.
DR1,DR4w14,DR4w15	5'	CTC	CTC	GAG	CAG	AGG	CGG	GCC	GCG	3'	63
DR2*	5'	T--	---	---	G-C	---	--C	---	---	3'	64
DR3	5'	---	---	---	---	-A-	---	-G-	CG-	3'	65
DR4w4	5'	---	---	---	---	-A-	---	---	---	3'	66
DR4w13	5'	---	---	---	---	---	---	---	-A-	3'	67
DR5, DR6, DR4w10	5'	A--	---	--A	G-C	GA-	---	---	---	3'	68
DR7	5'	A--	---	---	G-C	---	---	-G-	CA-	3'	69
DR8	5'	T--	---	--A	G-C	---	---	---	CT-	3'	70
DR9	5'	T--	---	---	-G-	---	---	---	-A-	3'	71

DRB3 Genes

											SEQ ID NO.
DR2	5'	A--	---	---	---	GC-	---	---	---	3'	72
DR3	5'	---	---	---	---	-A-	---	-G-	CAG	3'	73
DR7, DR9	5'	---	---	---	-G-	---	---	---	-A-	3'	74

Patient	HLA-DR
1008	1,4w4
1012	1,3
1013	1,7
1014	1,4w4
1015	4w4,4w4

* The dashes refer to amino acids in common with DR1, DR4w14, DR4w15

T cell receptors containing Vβ17 or fragments thereof which are immunogenic or can be made immunogenic can be used to immunize human subjects by methods

demonstrated by Example VIII. Such immunizations can result in an effective immune response.

EXAMPLE XI

Synovial tissue specimens were obtained from
5 proven RA patients undergoing joint replacement surgery. HLA-DR analysis was conducted as described in Example IX(D).

A. Polymerase chain reaction (PCR) amplification of T cell receptor β-chain genes

10 T cell receptor β-chain genes were amplified in two-stage amplification reactions with nested pairs of HPLC-purified oligonucleotide primers (Midland Certified Reagents, Midland, TX.) shown in Figure 3. RNAs were reverse transcribed (1 hour, 42°C) with the Cβext primer
15 (40 pmol) in 12 μl of reaction buffer (Hart et al., Lancet ii:596-599 (1988)). Reactions were diluted with a master mix (8 μl) containing the Vβcons primer (40 pmol), nucleotides and reaction buffer minus $MgCl_2$ (final Mg^{+2} concentration= 3.6 mM). Samples were denatured (15
20 minutes, 95°C) and 20 cycles of PCR were conducted using Taq polymerase (1 unit, Cetus Ampli-taq). Each cycle consisted of a 1 minute denaturation at 95°C, a two minute annealing step and a two minute extension at 72°C. The first two cycles were annealed at 37°C and 45°C and the
25 remainder at 50°C. One microliter aliquots of these Stage I reactions were added to 100 μl Stage II amplification reactions (Cetus Gene-Amp Kit) containing the Cβint primer (100 pmol) and either the Vβ8, Vβ12, Vβ17, or 5'Cβ primers (100 pmol) or the Vβcons primer (100-700 pmol).
30 Stage II amplifications were conducted for the indicated number of cycles with a 50°C annealing temperature and without the 37°C and 45°C ramping.

56

B.   VB17 T cells are cytotoxic for synovial adherent cells

Two parallel cultures were established from single cell suspensions, derived by enzymatic digestion
5 as described below from the synovial tissue of patient 1008. The first, a bulk culture of total synovial cells, was plated at 2 x 10^6/ml in RPMI 1640, 5% FBS, 20% HL-1 (Ventrex Laboratories, Portland, ME), 25mM HEPES, glutamine and antibiotics. The culture was grown
10 undisturbed for two weeks in the absence of exogenous antigen or growth factors. The second, a synovial cell monolayer culture, was initiated as above and regularly trypsinized and passaged during this two-week period. Monolayer cells were plated at 5 x 10^4 cells/well in flat-
15 bottomed, 96-well microtiter plates and cultured overnight. Non-adherent cells from the total synovial cell culture were then plated at 10 cells/well onto the monolayers. Wells positive for T cell growth were expanded and adapted to culture by regular stimulation
20 with autologous synovial monolayers for 3 days in media without IL-2 followed by a 4 day culture in medium containing 20% supernatant from lymphokine activated killer (LAK) cells, a source of IL-2 (Allegretta et al., _Science_ 247:718-721 (1990)). Later passages were adapted
25 to weekly stimulation with allogeneic PBLs and anti-CD3 antibody (Coulter Immunology, Hialeah, FL) in place of synovial cell monolayers.

Cytotoxicity assays were conducted as described Stedman et al., _Immunol. Meth._ 119:291-294 (1989),
30 incorporated herein by reference, using ^{35}S-labeled synovial monolayer cells or EBV-transformed B cells as targets. Target cells were labeled overnight, trypsinized (adherent cells only), washed and plated at 2000 cells per well in 96-well round bottom microtiter
35 plates. T cells were activated with allogenic PBLs and anti-CD3 antibody in medium containing LAK supernatant

for 7 days prior to the assay and added to the targets at
the indicated effector:target ratios. Cultures were
incubated overnight at 37°C, centrifuged at 300xg for 2
minutes and radioactivity in 50 μl of the supernatant
5   quantified.

The per cent specific lysis was calculated
relative to detergent-lysed targets as described in
Townsend et al., _Cell_ 44:959-968 (1986), incorporated
herein by reference. At effector:target ratios of 1.0,
10 2.5 and 5.0, the percent lysis of synovial adherent cells
by 1008.8 T cells was approximately 4%, 14% and 33%,
respectively. By comparison, at the same effector:target
ratios, 1008.8 T cells had no demonstrated effect on EBV-
transformed B cell targets. Similarly, at the same
15 effector:target ratios, the percent lysis of synovial
adherent cells by MS3 cells was approximately 0%, 0% and
3%, respectively.

T cells were isolated from the synovial tissue
of patient 1008 by co-cultivation with synovial cell
20 monolayers. Since the antigens recognized by pathogenic
T cells in RA are unknown, synovial cell monolayers were
used as stimulators of in vitro synovial T cell growth.
The relevant target cells were assumed to be present in a
bulk adherent cell culture from diseased synovium. Of
25 192 microwell co-cultures plated, 7 were positive for T
cell growth within 10-14 days. T cells were expanded and
maintained in vitro by alternately stimulating with
synovial cell monolayers and medium containing LAK
supernatant. Flow cytometry revealed that each of these
30 seven cultures was 100% CD4+. One culture designated
1008.8, grew especially vigorously and, as assessed
microscopically promoted the destruction of the monolayer
cells. CTL assays confirmed the cytotoxicity of 1008.8
for these monolayer targets as discussed above.
35 Cytolysis was specific for synovial cell targets, as no

lysis of autologous Epstein-Barr virus-transformed B cells was demonstrable. Neither of the targets was lysed by a CD4+, myelin basic protein-reactive human CTL clone, MS3, excluding the possibility that the monolayer cells

5 were susceptible to lysis by any activated T cell. In repeated assays with 1008.8, specific lysis never exceeded 30-35%, suggesting that the relevant synovial target cell comprises approximately that proportion of the total monolayer culture, which based on morphology is

10 a mixture of multiple cell types.

The T cell receptor (TCR) ß-chain gene of 1008.8 was amplified by the polymerase chain reaction (PCR) as described in Mullis et al., <u>Meth. Enzym.</u> 155:335-350 (1987), incorporated herein by reference, and

15 its DNA sequence was determined. Amplification was accomplished using a consensus Vß primer (Vßcons), a degenerate 16 nucleotide primer (n=256) which is homologous at all 16 residues with 78%, and at 15 of 16 residues with 98%, of known human TCR Vß genes, which

20 have been compiled by Kimura et al., <u>J. Immol.</u> 17:375-383 (1987). This primer was designed to amplify ß-chain rearrangements containing any of the known Vß genes, thus allowing analysis of T cell clones for which <u>a priori</u> knowledge of Vß gene usage is unavailable. Sequencing of

25 the Vßcons-amplified ß-chain gene of 1008.8 revealed a single Vß17-Jß1.1 rearrangement as shown in Table 16. Subsequently, the remaining six cultures derived from patient 1008 were analyzed by PCR amplification using a Vß17-specific primer shown in Table 13. TCR genes from

30 three of those six were amplifiable, indicating that, of the 7 T cell cultures obtained from the synovium of this patient, 4 had rearranged and expressed the Vß17 gene.

59

C. Vβ17 T cells are enriched among activated T cells in RA synovium

Synovial tissue was digested with agitation for
4 hrs at 37°C in RPMI-1640 and 10% fetal bovine serum
5   (FBS) containing 4 mg/ml collagenase (Worthington
Biochemicals, Freehold, NJ) and 0.15 mg/ml DNAse (Sigma
Chemical, St. Louis, MO). Digests were passed through an
80-mesh screen and single cells were collected from the
interface of Ficoll density gradients, washed and
10   incubated at 10^6/ml for 30 min at 0°C with 5 µg/ml control
mouse IgG (Coulter) in PBS containing 2% BSA. Cells were
washed 3X and incubated for 30 min at 0°C with magnetic
beads conjugated to goat anti-mouse IgG (Advanced
Magnetics, Cambridge, MA). After magnetic removal of the
15   beads, the remaining cells were incubated 30 minutes at
0°C with 5 µg/ml mouse anti-human IL-2R (Coulter), washed
and selected with magnetic beads as above. Cell-coated
beads from the mIgG preadsorption and the IL-2R antibody
selection were washed 3X, immediately resuspended in
20   acidified-guanidinium-phenol-chloroform and RNA was
prepared as described in Chomczynski et al., Anal.
Biochem. 162:156-159 (1987).

RNAs from magnetic bead preparations were
reverse transcribed and amplified for 20 cycles in stage
25   I reactions with the Vβcons and Cβext primers. One µl of
each reaction was reamplified for 20 cycles in individual
stage II reactions containing the Cβint primer in
conjunction with the Vβ17, Vβ8, Vβ12 and 5'Cβ primers.
Aliquots of each reaction were diluted in 20X SSC,
30   denatured by boiling and chilled in an ice slurry.
Samples were loaded onto nitrocellulose membranes,
hybridized to a human TCR β-chain constant region probe
and washed with 0.1X SSC, 0.1% SDS at 56°C. Bound
radioactivity was quantified by liquid scintillation
35   spectroscopy. The amounts of product produced by 40
total cycles with each of the respective primer

combinations falls in the linear portion of a product versus cycle number quantification curve. Values shown in Table 17 below reflect the relative increase or decrease for the specific Vßs in the IL2-R+ versus mIgG

5   controls calculated according to the formula:

$$\frac{\text{specific V}\beta \text{ cpms (IL2-R+)}}{\text{specific V}\beta \text{ cpms (mIgG)}} \bigg/ \frac{\text{C}\beta \text{ cpms (IL2-R+)}}{\text{C}\beta \text{ cpms (mIgG)}}.$$

TABLE 17

		Ratio $\frac{\text{IL-2R+}}{\text{mIgG}}$		
Sample Experiment #		VB17	VB8	VB12
1012				
	1	1.88	0.42	1.35
	2	1.60	0.39	0.72
	3	2.11	0.48	0.64
X⁻ ±S.D.		1.86 ±0.25	0.43 ±0.04	0.900 ±0.38
1013				
	1	2.54	0.49	N.D.
	2	3.65	0.87	0.87
	3	4.29	2.07	0.96
X⁻ ±S.D.		3.49 ±0.88	1.14 ±0.82	0.91 ±0.06
1014				
	1	4.70	0.17	N.D.
	2	1.68	0.10	1.50
	3	1.92	0.09	0.44
	4	2.34	0.07	0.55
X⁻ ±S.D.		2.66 ±1.38	0.10 ±0.04	0.83 ±0.58
1015				
	1	3.40	0.20	0.85
	2	2.85	0.47	1.82
X⁻ ±S.D.		3.12 ±0.50	0.38 ±0.15	1.33 ±0.68

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Next, the presence of Vß17 T cells in the
synovial tissue of other RA patients was determined.
Since the rheumatoid synovium contains a mixture of
activated and non-activated T cells, the activated T
5   cells were identified as the most relevant for the
initiation and perpetuation of the disease pathogenesis.
Thus, activated T cells from single-cell suspensions of
synovial tissue were selected using magnetic beads and
antibodies reactive with the human interleukin-2 receptor
10   (IL-2R). Cell suspensions from each patient were
pretreated with an isotype-matched mouse IgG (mIgG) and
magnetic beads to control for non-specific adsorption.
RNAs were directly extracted from cells in the IL-2R+ and
control samples without _in vitro_ culture and, therefore,
15   are expected to accurately reflect T cell distributions
in synovial tissue at the time of surgical removal.

The initial PCR amplifications of these
magnetic bead RNA preparations revealed greater amounts
of Vß17 PCR product in the IL2-R+ samples than in the
20   corresponding mIgG controls. The presence of TCR mRNA in
the mIgG samples indicates that T cells non-specifically
adhered to the magnetic beads. The apparent increase in
the IL2-R+ sample suggests that the activated T cell
compartment contained more Vß17 T cells than the
25   unselected synovial T cell compartment. Thus, a
quantitative PCR analysis was used to formally examine
the relative proportions of Vß17 T cells in the IL-2R+
and mIgG control samples (Table 17). Magnetic bead RNAs
were reverse transcribed, preamplified with Vßcon and
30   Cßext and reamplified in separate reactions with a
constant region primer (Cßint) and each of the Vß-
specific primers, Vß17, Vß8 and Vß12. The second stage
amplification was also performed with two Cß primers
(5'Cß and Cßint) in order to estimate the total ß-chain
35   present in each sample and to provide benchmarks for
normalizing the results of specific Vß quantification in

the respective IL-2R+ and control sample pairs. The
proportion of Vβ17 DNA, relative to total Cβ, was
increased in the IL-2R+ samples over that found in the
mIgG control samples for each of the 5 patients examined.

5   This increase was observed in multiple analyses and the
means are shown in Table 17. Enrichment was not a
product of the isolation procedure, since the quantity of
Vβ8 or Vβ12 DNA amplified was not significantly increased
in the IL-2R+ fraction of any of the patients. Thus,

10   activated T cells in the rheumatoid synovium do not
represent a cross section of all possible Vβ families,
but preferentially contain Vβ17 T cells, and possibly
other Vβ families which were not quantified in this
analysis.

15   **D. Synovial Vβ17 T cells display limited heterogeneity**
             RNAs were reverse transcribed and amplified
with the Vβcons and Cβext primers in 20 cycle stage I
reactions and with the Cβint and Vβcons (1008.8) or Vβ8,
Vβ12, and Vβ17 primers (magnetic bead RNA preparations)

20   in 35 cycle stage II reactions. Double stranded reaction
products were electrophoresed in 2% Nu-Sieve agarose
gels. After purification from gel slices with Gene Clean
(Bio 101, San Diego, CA), samples were base denatured and
either directly sequenced or cloned into plasmid for

25   sequencing of multiple independent rearrangements. In
all cases, samples were sequenced from the Cβseq primer
(Figure 2) with T7 polymerase (Sequenase, United States
Biochemicals, Cleveland, OH).

             Vβ17 rearrangements present in the IL-2R+ RNAs

30   were amplified from the Vβcons and Cβext preamplification
with the Vβ17 and Cβint primer pair and the reaction
products sequenced. PCR products from patients 1014 and
1015 were directly sequenced and the results obtained
were consistent with the presence of a single Vβ17

35   rearrangement in each of these amplified samples (Table

17). The PCR product of patient 1013 was cloned into plasmid and the thirteen isolates that were sequenced contained identical VB17 rearrangements. Sequencing of plasmid clones from patient 1012 revealed the presence of

5 two dominant rearrangements (5 isolates of each) and a single isolate of another. Thus, the VB17 repertoire in the RA synovium is of limited heterogeneity, indicative of clonal or oligoclonal expansion of VB17 T cells _in vivo_. This is in contrast to the VB8 and VB12

10 repertoires, from these same synovial preparations, which showed significant heterogeneity. None of the PCR-amplified VB8 and VB12 samples analyzed were directly sequenceable and plasmid cloning of VB8 rearrangements from patient 1012 revealed 4 different sequences in 5

15 clones analyzed.

VB17 rearrangements in PBLs from patient 1012 also revealed greater diversity than that seen in the synovial IL-2R+ sample. RNA from a 3 day culture of PHA/PMA stimulated 1012 PBLs was amplified with the VB17

20 primer, as for the synovial sample, and the products cloned into plasmid. Nine different rearrangements, none of which corresponded to those present in the 1012 synovium, were found in the 10 clones that were sequenced. Thus, the restricted heterogeneity of VB17

25 rearrangements in the activated synovial T cell population of patient 1012, as well as the other patients examined, likely results, not from random T cell trafficking, but from the selective expansion of those VB17-bearing T cells in the diseased tissue.

EXAMPLE XII

A. Detection of TCR β-chain transcripts in synovial T cells using Vβ-specific PCR-amplification

T cell receptor β-chain genes were amplified in
5 two-stage reactions with individual Vβ-specific primers
as described in Wucherpennig et al., Science, 248:1016-
1019 (1990), incorporated herein by reference, and nested
Cβ primers. RNAs were reverse transcribed (1 hour, 42°C)
with the Cβext primer (40 pmol) in 12 μl of reaction
10 buffer (Hart et al., supra). Reactions were diluted with
a master mix (8 μl) containing nucleotides, reaction
buffer minus $MgCl_2$ (final Mg^{+2} concentration = 3.6 mM) and
Taq polymerase, apportioned among 19 tubes containing the
individual Vβ primers. Samples were denatured (15
15 minutes, 95°C) and 20 cycles of PCR were conducted. Each
cycle consisted of a one minute denaturation at 95°C, a
two minute annealing step and a two minute extension at
72°C. The first two cycles were annealed at 37°C and
45°C and the remainder at 50°C. One microliter aliquots
20 of these Stage I reactions were added to 100 μl Stage II
amplification reactions (Cetus Gene-Amp Kit) containing
the Cβint primer (100 pmol) and 100 pmol of the Vβ
primers used in the corresponding preamplifications.
Stage II amplifications were conducted for 20 cycles with
25 a 50°C annealing temperature and without the 37°C and
45°C ramping. Five μl of each reaction was
electrophoresed in 2% agarose gels, transferred to
nitrocellulose and hybridized to a ^{32}P-labeled β-chain
constant region probe. Blots were exposed to X-ray film
30 and scored for the presence or absence of Vβ-specific
amplification.

B. VβB17, Vβ814, Vβ9 and Vβ3 transcripts are common among
activated synovial T cells

     To ensure that the prevalence of Vβ17
rearrangements in the earlier studies did not result from
an amplification bias of the Vβ16mer primer, and to
assess the presence of other Vβ genes in the synovium,
TCR transcripts in the IL2-R+ samples were analyzed with
a panel of 19 PCR primers, specific for known human Vβ
gene families (Table 18). The Vβcons primer of Example
XI is equivalent to the Vβ16mer primer. The number of Vβ
genes detectable in these samples was variable, ranging
from two to twelve. Vβ17 was found in four of the five
patients, confirming the previous analyses using the
Vβ16mer primer. Vβ14 transcripts also were found in four
of the five patients and Vβ3 and Vβ9 transcripts were
detectable in three of the five samples. Thus, T cells
bearing these 3 Vβ polypeptides may also contribute to
synovial inflammation.

TABLE 18

Analysis of IL-2R+ Synovial T Cells with
Individual Vβ-specific Primers

VB Families

Patient #	1	2	3	4	5	6	7	8	9	10	11	12	14	15	16	17	18	19	20
1012			+	+												+			
1013						+			+			+							
1014												+				+			
1015			+			+	+	+			+	+	+	+		+	+	+	
1020	+	+	+		+				+				+	+		+	+	+	

EXAMPLE XIII

Vaccination Against EAE with a CDR4 Peptide of Vβ8.2

Rats were immunized with 100 μg of a synthetic peptide containing the sequence VPNGYKVSRPSQGDFFLTL (SEQ
5   ID No.46) found in the fourth hypervariable or CDR4 region of the rat TCR β chain identified as Vβ8.2.   The peptide, dissolved in saline, was emulsified in an equal volume of complete Freund's adjuvant (CFA) containing 10 mg/ul of mycobacterial tuberculosis.   The animals were
10 challenged 30 days later with 50 μg of guinea pig myelin basic protein in CFA.   The CDR4 peptide vaccination resulted in a reduced incidence of disease as well as a reduced severity as measured both clinically and histologically as shown in Table 19.   The histology was
15 performed essentially as described in Hughes & Powell, J. Neuropath. Exp. Neurol. 43:154 (1984), which is incorporated herein by reference.

TABLE 19

Protection from EAE in the Rat by Vaccination with a TCR-CDR4 Peptide

Vaccination	Challenge	#Clinical Signs #Tested	Mean Max Severity[2]	Mean Onset	Mean Duration	#With Histology #Tested	Mean Histology Score
Rat CDR4 in CFA	50 μg MBP/CFA	6/10	1.5	11.8	5.4	3/10	0.6
PBS/CFA	50 μg MBP/CFA	9/10	2.5	12.1	6.1	6/9	1.4
None	50 μg MBP/CFA	10/10	3.0	10.5	7.3	9/9	3.0

[1] rat CDR4 peptide sequence: VPNGYKVSRPSQGDFFLTL (SEQ ID No.46)

[2] graded on a 3-point scale as described in Example I

[3] graded on a 4-point scale

Although the invention has been described with reference to the presently-preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention.

5   Accordingly, the invention is limited only by the following claims.

WE CLAIM:

1. A vaccine for preventing or treating a T cell mediated pathology in a vertebrate, comprising an immunogenically effective amount of at least one substantially pure T cell receptor, or a fragment
5   thereof, corresponding to a T cell receptor present on the surface of T cells mediating said pathology, and a pharmaceutically acceptable medium.

2. The vaccine of claim 1, wherein said T cell receptor or fragment thereof comprises substantially an
10   amino acid sequence of Vß3, Vß14, Vß17 or any combination thereof.

3. The vaccine of claim 1, wherein said T cell receptor comprises the amino acid sequence of Vß17.

4. The vaccine of claim 3, wherein said T cell mediated pathology is rheumatoid arthritis.

5. The vaccine of claim 1, wherein said fragment comprises a variable region sequence of said T cell receptor.

6. The vaccine of claim 5, wherein said variable region sequence is the ß-chain variable region.

7. The vaccine of claim 6, wherein said ß-chain variable region comprises substantially the amino acid sequence of Vß17 or fragment thereof.

8. The vaccine of claim 7, wherein said ß-chain variable region comprises substantially the sequence SQIVNDFQK.

9. The vaccine of claim 6, wherein said ß-chain variable region comprises substantially the amino acid sequence of Vß14 or fragment thereof.

10. The vaccine of claim 9, wherein said ß-chain variable region comprises substantially the sequence SMNVEVTDK.

11. The vaccine of claim 6, wherein said ß-chain variable region comprises substantially the amino acid sequence of Vß3 or fragment thereof.

12. The vaccine of claim 11, wherein said ß-chain variable region comprises substantially the amino acid sequence SYDVKMKEK.

13. The vaccine of claim 1, wherein said fragment comprises a V(D)J junctional sequence.

14. The vaccine of claim 1, wherein said fragment comprises a joining (J) region sequence.

15. The vaccine of claim 1, further comprising an adjuvant.

16. The vaccine of claim 1, wherein said vaccine comprises more than one type of T cell receptor or fragment thereof.

17. The vaccine of claim 16, wherein the T cell receptors or fragments thereof are selected from any combination of Vß3, Vß14 or Vß17.

18. The vaccine of claim 1, wherein said vaccine comprises more than one fragment corresponding to different sequences of the same T cell receptor.

19. The vaccine of claim 18, wherein said fragments are selected from any combination of Vβ3, Vβ14 or Vβ17.

20. The vaccine of claim 1, wherein said TCR fragment comprises a substantially pure peptide from a TCR β-chain region reactive with a superantigen associated with said T cell pathology.

21. The vaccine of claim 20, wherein said substantially pure peptide has an amino acid sequence of $X_1-X_2-E-X_3$, wherein X_1 and X_3 are each a single amino acid or a group of amino acids, and X_2 is R or K.

22. The vaccine of claim 21, wherein said amino acid sequence is substantially the sequence EGYSVSREKKESFPL.

23. The vaccine of claim 21, wherein said amino acid sequence is substantially the sequence EGYKVSRKEKRNFPL.

24. The vaccine of claim 21, wherein said amino acid sequence is substantially the sequence EGYSVSREKKERFSL.

25. The vaccine of claim 1, wherein said fragment is conjugated to a carrier.

26. The vaccine of claim 1, wherein said T cell mediated pathology is multiple sclerosis, said vertebrate is a human, and said fragment comprises substantially the sequence SGDQGGNE so as to elicit an
5 immune response against T cell receptors having substantially the sequence SGDQGGNE.

27. The vaccine of claim 1, wherein said T cell receptor comprises the sequence SGDQGGNE.

28. A method of vaccinating an individual exhibiting or at risk of exhibiting a T cell-mediated pathology, comprising administering to the individual the vaccine of claim 1.

29. The method of claim 28, wherein said vaccine is administered more than once.

30. The method of claim 28, wherein said vaccine is administered in a formulation including an adjuvant.

31. A method of treating unregulated T cell clonal replication in an individual, comprising administering the vaccine of claim 1 to the individual.

32. A method of selecting a vaccine for use in treating a T cell mediated pathology comprising the steps of:

    a. obtaining T cell clones mediating said pathology;

5

    b. determining the amino acid sequence of T cell receptors or fragments thereof from T cell clones associated with said pathology;

    c. selecting segments of those T cell receptors which are characteristic of said associated T cell receptors but not of non-associated T cell receptors; and

10

    d. selecting amino acid sequences of said selected sequences which are capable of eliciting an immunogenic response to said T cell receptor, and thereby selecting the vaccine.

15

33.   A method of diagnosing or predicting susceptibility to a T cell mediated pathology in an individual comprising detecting T cells having a β-chain variable region of Vβ3, Vβ14 or Vβ17 or a fragment

5 thereof in a sample from the individual, the presence of abnormal expression of said Vβ-containing T cells indicating said pathology or susceptibility to said pathology.

34.   The method of claim 33, wherein said T cells have the β-chain variable region of Vβ17 or a fragment thereof.

35.   The method of claim 33, wherein said pathology is rheumatoid arthritis.

36.   The method of claim 34, comprising detecting a portion of said Vβ17 which substantially does not occur on non-T cell mediated pathology associated T-cell receptors.

37.   The method of claim 33, wherein said sample is from synovial tissue.

38.   The method of claim 34, wherein said Vβ17 is detected by contacting said Vβ17 with a detectable ligand.

39.   The method of claim 34, wherein the presence of said Vβ17 is detected by a nucleotide probe which is complementary to the nucleotide sequence encoding Vβ17.

40. A method of preventing or treating a T cell mediated pathology, comprising inhibiting the binding of a T cell receptor to its binding partner to prevent proliferation of T cells associated with said T cell mediated pathology.

41. The method of claim 40, wherein said binding inhibition is effected by a ligand reactive with the T cell receptor binding partner.

42. The method of claim 41, wherein said ligand is an antibody.

43. The method of claim 40, wherein said binding inhibition is effected by a ligand reactive with a binding site on said T cell receptor.

44. The method of claim 41, wherein said ligand is an antibody.

45. The method of preventing or treating a T cell mediated pathology of claim 40, wherein said T cell receptor is a Vβ3-, Vβ14- or Vβ17-containing T-cell receptor.

46. The method of claim 45, wherein said T-cell receptor contains Vβ17.

47. The method of claim 45 wherein said pathology is rheumatoid arthritis.

48. The method of claim 45, wherein said binding partner is an HLA-DR predisposing for rheumatoid arthritis.

49. The method of claim 46, wherein attachment is prevented by binding a ligand to Vβ17.

50.   The method of claim 46, wherein attachment is prevented by binding a ligand to said Vβ17 binding partner.

51.   A method of preventing or treating a T cell mediated pathology in an individual comprising cytotoxically or cytostatically treating Vβ3-, Vβ14- or Vβ17-containing T-cells in the individual.

52.   The method of claim 51, wherein the T cells contain any combination of Vβ3, Vβ14 or Vβ17.

53.   The method of claim 51, wherein the T cells contain Vβ17.

54.   The method of claim 51, wherein the T cell mediated pathology is rheumatoid arthritis.

55.   The method of claim 53, wherein said Vβ17 containing T-cells are treated with a cytotoxic or cytostatic agent which selectively binds Vβ17.

56.   The method of claim 53, wherein said agent is an antibody attached to a moiety selected from the group consisting of radioactive moieties, chemotherapeutic moieties and chemotoxic moieties.

57.   A vaccine for preventing or treating a T cell mediated pathology in a vertebrate, comprising anti-idiotypic antibodies which are internal images of a T cell receptor or a fragment thereof corresponding to a
5 cell receptor present on the surface of a T cell mediating said pathology and a pharmaceutically acceptable medium.

58.   The vaccine of claim 57, wherein said fragment comprises a variable region sequence of the T cell receptor.

59.   The vaccine of claim 57, wherein said fragment comprises a V (D) J junction sequence.

60.   The vaccine of claim 57, further comprising an adjuvant.

61.   The vaccine of claim 57, wherein said vaccine comprises anti-idiotype antibodies which are internal images of more than one T cell receptor or fragment thereof.

62.   A method of vaccinating an individual exhibiting or at risk of exhibiting a T cell-mediated pathology comprising administering to the individual the vaccine of claim 57.

63.   The vaccine of claim 58, wherein said fragment comprises a junctional region sequence.

64.   A method of diagnosing or predicting susceptibility to multiple sclerosis in an individual comprising detecting T cells having substantially the sequence SGDQGGNE in a sample from the individual, the
5   presence of such T cells indicating multiple sclerosis or susceptibility to multiple sclerosis.

65.   The method of claim 64, wherein said sequence is detected by contacting said sequence with a detectable ligand.

66.   The method of claim 64, wherein the presence of said sequence is detected by a nucleotide probe which is complementary to the nucleotide sequence encoding said sequence.

67.   A method of preventing or treating multiple sclerosis comprising preventing the attachment of a T-cell receptor containing substantially the sequence SGDQGGNE to its binding partner.

68.   The method of claim 67, wherein attachment is prevented by binding a ligand to said sequence.

69.   The method of claim 67, wherein attachment is prevented by binding a ligand to said T cell receptor binding partner.

70.   A method of preventing or treating multiple sclerosis in an individual comprising cytotoxically or cytostatically treating T cells containing substantially the sequence SGDQGGNE in the individual.

71.   The method of claim 70, wherein said sequence containing T cells are treated with a cytotoxic or cytostatic agent which selectively binds said sequence.

72.   The method of claim 70, wherein said agent is an antibody attached to a moiety selected from the group consisting of a radioactive moieties and a chemotherapeutic moieties and chemotactic moieties.

73. A composition of matter comprising a substantially pure T cell receptor or a fragment thereof corresponding to a T cell receptor present on the surface of T cells mediating a pathology and an adjuvant.

74. A substantially pure peptide comprising substantially the sequence SGDQGGNE.

75. A substantially pure peptide from a TCR ß-chain region reactive with a superantigen associated with a T cell pathology.

76. The substantially pure peptide of claim 75, wherein said T cell pathology is rheumatoid arthritis.

77. A substantially pure peptide comprising substantially the amino acid sequence $X_1-X_2-E-X_3$, wherein X_1 and X_3 are each a single amino acid or a group of amino acids and, X_2 is R or K.

78. The substantially pure peptide of claim 77, wherein said amino acid sequence is substantially the sequence EGYSVSREKKESFPL.

79. The substantially pure peptide of claim 77, wherein said amino acid sequence is substantially the sequence EGYKVSRKEKRNFPL.

80. The substantially pure peptide of claim 77, wherein said amino acid sequence is substantially the sequence EGYSVSREKKERFSL.

79

81.   A vaccine for preventing or treating a T cell mediated pathology or an unregulated T cell clonal replication in a vertebrate, comprising an immunogenically effective amount of a T cell having a
5   receptor which comprises the β-chain variable region of Vβ3, Vβ14 or Vβ17.

82.   The vaccine of claim 81, wherein said β-chain variable region is Vβ17.

83.   The vaccine of claim 81, wherein said T cell mediated pathology is rheumatoid arthritis.

84.   A method of preventing or treating an individual exhibiting or at risk of exhibiting a T-cell mediated pathology, comprising administering to the individual the vaccine of claim 81.

85.   A vaccine for preventing or treating multiple sclerosis comprising an immunologically effective amount of a T cell having a receptor which comprises substantially the sequence SGDQGGNE.

86.   A method of preventing or treating multiple sclerosis comprising administering to an individual the vaccine of claim 85.

87.   A method of determining a T cell receptor binding partner associated with proliferation of T cells in a T cell mediated pathology, comprising:

(a)   identifying T cells that are a component
5 of the T cell mediated pathology;

(b)   determining a superantigen binding site of said T cells;

(c)   contacting a sample with said superantigen binding site; and

10   (d)   determining the binding of said T cell receptor binding partner to said superantigen binding site, wherein binding indicates the presence of said T cell receptor binding partner in the sample.

88.   The method of claim 87, further comprising administering to an individual an effective amount of said T cell receptor binding partner to prevent the proliferation of said T cells.

89.   The vaccine of claim 1, wherein said immunogenic fragment comprises substantially the amino acid sequence of the CDR2 region of said ß-chain variable region.

5   90.   The vaccine of claim 89, wherein said CDR2 region comprises substantially the sequence DPGLGLRLIYFSYDVKMKEKG (SEQ ID No.75) or an immunogenic portion thereof.

91.   The vaccine of claim 89, wherein said
10 CDR2 region comprises substantially the sequence DPGLGLRQIYYSMNVEVTDKG (SEQ ID No.76) or an immunogenic portion thereof.

92.   The vaccine of claim 89, wherein said
CDR2 region comprises substantially the sequence
DPGQGLRLIYYSQIVNKFQKG (SEQ ID No.77) or an immunogenic
portion thereof.

5   93.   The vaccine of claim 89, wherein said
pathology is rheumatoid arthritis.

94.   The vaccine of claim 93, wherein said
amino acid sequence comprises substantially the sequence
of Vβ3, Vβ14 or Vβ17.

10   95.   The vaccine of claim 89, wherein said
pathology is multiple sclerosis.

96.   The vaccine of claim 1, wherein said β-
chain VDJ region comprises substantially the sequence
ASSLGGAVSYN (SEQ ID No.3).

15   97.   The vaccine of claim 1, wherein said β-
chain VDJ region comprises substantially the sequence
ASSLGGEETQYF (SEQ ID No.4), ASSLGGFETQYF (SEQ ID No.5) or
ASSLGGTEAFF (SEQ ID No.6).

98.   The vaccine of claim 1, wherein said β-
20 chain VDJ region comprises substantially the sequence
CAIGSNTE (SEQ ID No. 2).

99.   A method of preventing or treating a T
cell-mediated pathology in an individual, comprising
administering to the individual a nucleic acid encoding a
T cell receptor or an immunogenic fragment thereof in a
5   form capable of being expressed in said individual.

100.   The method of claim 99, wherein said
nucleic acid is DNA.

101.   The method of claim 99, wherein said nucleic acid is RNA.

102.   The method of claim 99, wherein said nucleic acid is administered into a muscle of the individual.

103.   The method of claim 99, wherein said immunogenic fragment comprises the amino acid sequence of a ß-chain V(D)J region of said T cell receptor.

104.   The method of claim 103, wherein an amino acid sequence is substantially the amino acid sequence of Vß3, Vß4, Vß12, Vß14 or Vß17.

105.   The method of claim 104, wherein said pathology is arthritis or multiple sclerosis.

106.   The method of claim 99, wherein said immunogenic fragment comprises the amino acid sequence of a variable region of said T cell receptor.

107.   The method of claim 106, wherein said variable region is a ß-chain variable region.

108.   The method of claim 107, wherein said amino acid sequence comprises substantially the sequence of the CDR2 region of said ß-chain variable region.

109.   The method of claim 107, wherein said amino acid sequence comprises substantially the sequence of the superantigen binding region of said ß-chain variable region.

110.   The method of claim 107, wherein said ß-chain variable region is Vß3, Vß4, Vß12, Vß14 or Vß17.

111. The method of claim 106, wherein said pathology is rheumatoid arthritis or multiple sclerosis.

112. A vector containing the nucleic acid of claim 99.

113. A composition comprising the vector of claim 99 and a pharmaceutically acceptable medium.

FIG.1

	CDR1		CDR2		CDR4
Vβ3	FLAVGLVDVKVTQSSRYLVKRTGEKVFLECVQDMDHENMFWYRQDPGLGLRLIYFSYDVKMKEKGDIPEGYSVSREKKERFSLILESASTNQTSMYLCASS
Vβ14	MGPQLLGYVVLCLLGAGPLEAQVTQNPRYLITVTGKKLTVTCSQNMNHEYMSWYRQDPGLGLRQIYYSMNVEVTDKGDVPEGYKVSRKEKRNFPLILESPSPNQTSLYFCASS
Vβ17	MSNQVLCCVVLCFLGANTVDGGITQSPKYLFRKEGQNVTLSCEQNLNHDAMYWYRQDPGGGLRLIYYSQIVNDFQKGDIAEGYSVSREKKESFPLTVTSAQKNPTAFYLCASS

FIG. 2A

```
                        V_____    D   J_____   C_____
                                                                    3'
5'_____
                                     3'___5'  3'___5'  3'___5'
                                     CβseQ    Cβint    Cβext


     Vβcons      Vβ17        Vβ8/V 12        5'Cβ
     5'___3'   5'___3'     5'___3'        5'___3'
3'_____
                                                                    5'
```

FIG. 2B

```
                  G AC         CAAA
Vβcons    5' T TC TGGTA     CA 3'
             T TT      TCGT

Vβ17      5' TCACAGATAGTAAATGACTTTCAG 3'

Vβ8       5' TCTCCACTCTGAAGATCC 3'

Vβ12      5' GATTTCCTCCTCACTCTG 3'

5'C       5' CAAGCTGTTCCCACCCGA 3'

Cβext     5' CCAGAAGGTGGCCGAGAC 3'

Cβint     5' GCGGCTGCTCAGGCAGTA 3'

Cβseq     5' CGACCTCGGGTGGGAACA 3'
```
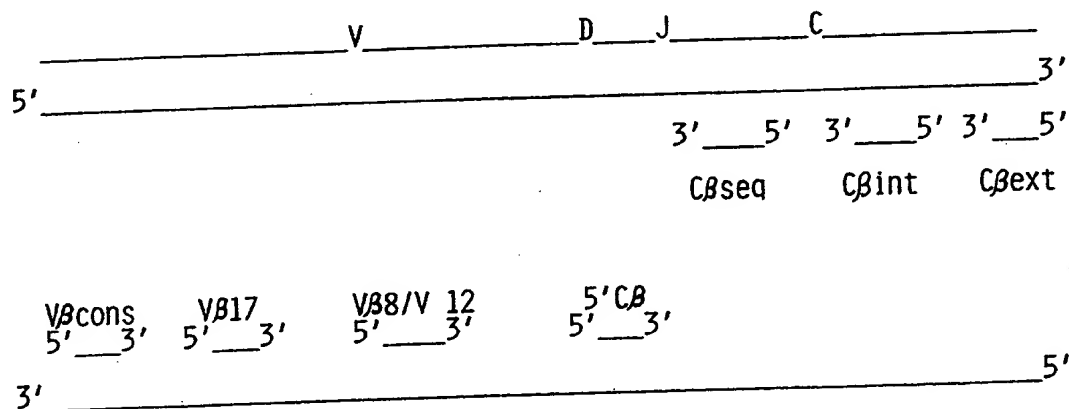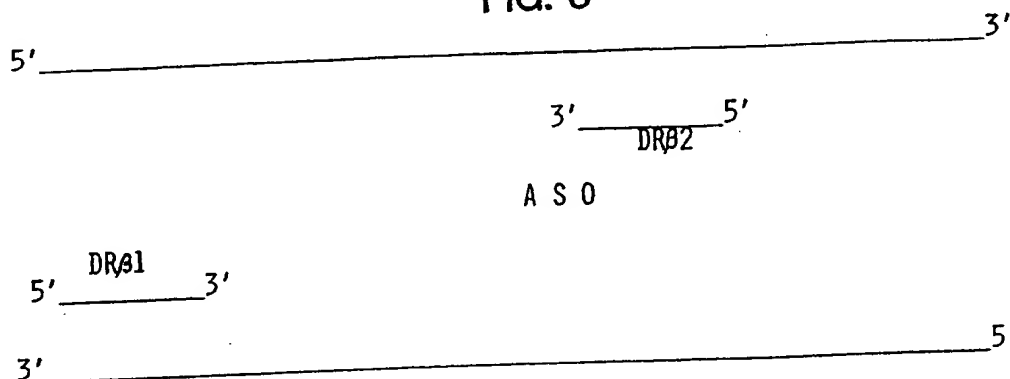
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FIG. 3

$$5' \rule{9cm}{0.4pt} 3'$$

$$3' \underline{\quad\quad} 5'$$
$$DR\beta2$$

A S O

$$DR\beta1$$
$$5' \underline{\quad\quad} 3'$$

$$3' \rule{9cm}{0.4pt} 5'$$

DRβ1   5'   G A $\overset{A}{\underset{C}{G}}$ T C T G G A A C A G C   3'

DRβ2   5'   G T A G T $\overset{A}{\underset{G}{T}}$ G T T C T G C A   3'

HLA-DR ALLELE-SPECIFIC OLIGONUCLEOTIDES

DRβ1 Genes

		CTC	CTC	GAG	CAG	AGG	CGG	GCC	GCG	
DR1,DR4w14,DR4w15	5'	CTC	CTC	GAG	CAG	AGG	CGG	GCC	GCG	3'
DR2	5'	T--	---	---	G-C	---	--C	---	---	3'
DR3	5'	---	---	---	---	-A-	---	-G-	CG-	3'
DR4w4	5'	---	---	---	---	-A-	---	---	---	3'
DR4w13	5'	---	---	---	---	---	---	---	-A-	3'
DR5, DR6, DR4w10	5'	A--	---	--A	G-C	GA-	---	---	---	3'
DR7	5'	A--	---	---	G-C	---	---	-G-	CA-	3'
DR8	5'	T--	---	--A	G-C	---	---	---	CT-	3'
DR9	5'	T--	---	---	-G-	---	---	---	-A-	3'

DRB3 Genes

DR2	5'	A--	---	---	---	GC-	---	---	---	3'
DR3	5'	---	---	---	---	-A-	---	-G-	CAG	3'
DR7, DR9	5'	---	---	---	-G-	---	---	---	-A-	3'

Patient	HLA-DR
1008	1,4w4
1012	1,3
1013	1,7
1014	1,4w4
1015	4w4,4w4

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